PHARMACOKINETIC EVALUATION OF A GASTROINTESTINAL MOTILITY TRETMENT OF DRUG IN HEALTHY HUMAN VOLUNTEERS (CINITAPRIDE)

Thesis Submitted *to*

The Tamilnadu Dr.M.G.R Medical University, Chennai

In partial fulfillment of the requirements

for the award of the Degree of

***M*ASTER OF PHARMACY**

IN

PHARMACOLOGY

Submitted by

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SEPTEMBER – 2009

CERTIFICATE

This is to certify that project work entitled '**Pharmacokinetic evaluation of Gastro intestinal motility treatment of a drug in Human volunteers (Cinitapride)'** is done by **Mr.G.SAMBASIVA RAO** in partial Fulfillment of the requirement for the award of Master of Pharmacy in carried out at Pharmacology was carried out at **Cadila Healthcare Ltd.,(PTC),Sarkhej Bavla N.H.NO.8A, Moraiya, Tal. Sanand, Dist.Ahmedabad – 382 210** and in R.V.S.College of Pharmaceutical Science, Sulur, Coimbatore.Which is affiliated to **Dr.M.G.R.Medical University, Chennai**, under guidance of **Mr.Jitendra Parmar**, Head of the Pharmacokinetic Department and under the guidance of **Mr.B.Suresh** Lecturer Of Pharmacology Department, R V S Sulur , Coimbatore.

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Place: Coimbatore

Date:

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As required by university regulation, I wish to state that this work embodied in this thesis titled **"Pharmacokinetic evaluation of Gastro intestinal motility treatment of a drug in Human volunteers (Cinitapride)"** forms my own contribution to the research work carried out under the guidance of **Mr. Suresh** and of **Mr.Jitendra Parmar**, Changela. This work has not been submitted for any other degree of this or any other university. When ever references have been made to previous work of others, it has been clearly indicated as such and included in the bibliography.

Signature of the Candidate

Mr.G.Sambasiva Rao

DEDICATED TO…….

MY BELOVED PARENTS &

SISTER

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In the name of God, the almighty, the most generous, the most merciful and beneficiary, My constant guide, source of strength and support.

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(G.Sambasiva Rao)

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AIM AND OBJECTIVE

The basic aim of this project is to conduct the Bioequivalence study of an Antidepressant, in accordance with the regulatory authority. The BE study is conducted on a test product, 'T'and reference product 'R'. 'T', and 'R'are oral Tablet containing drug Cinitapride. In a dose of 150mg. Drug Cinitapride is an Gastrointestinal motility treatment drug.

The study objectives included:

- Assessment of the bioavailability of test product A while comparing with a reference product B in 6 normal, adult, human subjects under fasting condition. The bioequivalence assessed is to be assessed under following pharmacokinetic Parameters:
  - AUC_{0-t},
  - $AUC_{0-\infty}$,
  - C_{max},
  - T_{max},
  - K_{el}, and
  - $t_{1/2.}$
- Monitoring of the adverse events and ensure safety of the subjects.

LIST OF ABBREVIATIONS

AE	**Adverse Event**
ANOVA	**Analysis of Variance**
ANDA	**Abbreviated new drug application**
AUC	**Area under the plasma concentration versus time curve**
$AUC\_{Extrapolated\ (\%)}$	**Percentage Area Under the Plasma Concentration extrapolated from AUC_{0-t} to $AUC_{0-\infty}$**
$AUC_{0-\infty}$	**The area under plasma concentration versus time curve from time zero to infinity**
AUC_{0-t}	**The area under the plasma concentration versus time curve from time zero to the last measurable concentration**
BA/BE	**Bioavailability / Bioequivalence**
BMI	**Body Mass Index**
CDSCO	**Central Drug Standard Control Organization**
cGMP	**Current good manufacturing practices**
CI	**Clinical Investigator**
C.I.	**Confidence interval**
C_{max}	**Concentration Maximum**
CPB	**Clinical Pharmacokinetics and Biopharmaceutics**
CRA	**Clinical Research Associate**
CRF	**Case Report Form**
CRF,	**Corticotrophin-releasing factor**
C.V.	**Coefficient of variance**
D	**Day**
Dept.	**Department**

CG	**Electro Cardiogram**
ELISA	**Enzyme linked immune sorbant assay**
FDA	**Food and drug administration**
XL	**Extended Release**
GCP	**Good Clinical Practice**
GGTP	**Gamma Glutamyl Transamino Peptidase**
GMP	**Good manufacturing**
HBsAg	**Hepatitis B surface Antigen**
HCT	**Hematocrit**
HCV	**Hepatitis C virus**
HCl	**Hydrochloride**
HIV	**Human Immuno Deficiency Virus**
Hr/Hrs	**Hours**
I.D	**Identity**
ICF	**Informed Consent Form**
ICH	**International Conference on Harmonization**
ICMR	**Indian Council of Medical Research**
IEC	**Independent Ethics Committee**
IP(s)	**Investigational Product(s)**
IL	**Immediate release**
K3EDTA	**Tri-potassium Ethylene Diamine Tetra Acetic acid**
K_{el}	**First order rate constant associated with the terminal (log-linear) portion of the curve**
Kg	**Kilogram**
LLOQ	**Lower limit of quantification**
LSM	**Least Square Means**
LC-MS-MS	**Tandem mass spectroscopy**

M	**Meter**
ME	**Medical Examination**
MR	**Modified Release**
Mg	**Milligram**
ml/mL	**Milliliter**
NA	**Noradrenaline**
ng.hr/mL	**Nanogram. Hour/milliliter**
No.	**Number**
PCV	**Packed Cell Volume**
PI	**Principal Investigator**
PK	**Pharmacokinetic**
PD	**Pharmacodynamic**
QC	**Quality control**
RLD	**Reference listed drug**
RPM	**Rotation per Minute**
SAE	**Serious Adverse event**
SAS	**Statistical Analysis Software**
SGOT	**Serum Glutamate Oxaloacetate Transaminase**
SGPT	**Serum Glutamate Pyruvate Transaminase**
SOP	**Standard Operating Procedure**
TrkB	**kinase-linked receptor**
$T_{1/2}$	**Elimination Half-life**
T_{max}	**Time at Concentration Maximum**
ULOQ	**Upper limit of Quantification**

1. INTRODUCTION

Bioavailability is used to describe the fraction of an administered dose of medication that reaches the systemic circulation, one of the principal properties of the drugs. By definition, when the drug is administered intravenously, its bioavailability is 100%. However when a medication is administered via other routes (such as by mouth), its bioavailability decreases (due to incomplete absorption and first-pass metabolism). Bioavailability is one of the essential tools in pharmacokinetics, as bioavailability must be considered when calculating dosages for non-intravenous routes of administration.

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 care costs is resulted in a tremendous 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 product.

This phenomenal growth of the generic pharmaceutical industry and the abundance of multisource products have prompted some questions among many health 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 bioequivalent 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.

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:

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

If the goal is to compare the two formulations of same drug then the experimental design should maintain the remaining factors constant. The resultant bioavailability may differ with respect to the amount absorbed, the rate of absorption or both. The bioavailability fraction f is the fraction of the administered dose that enters systemic circulation.

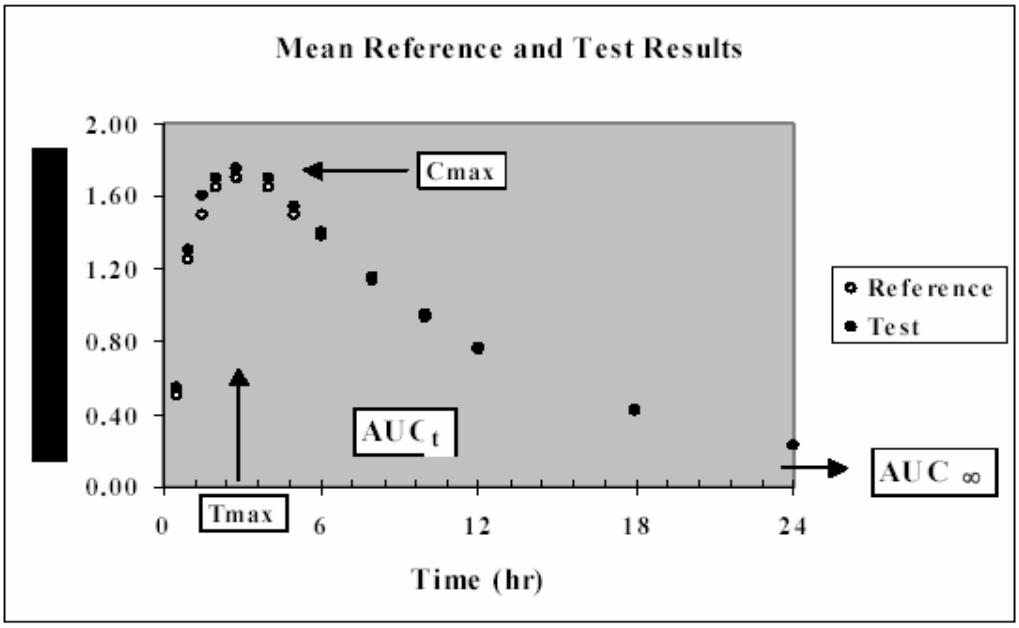
f = bioavailable dose / administered dose

Bioavailability reflects the extent of the systemic availability of the active therapeutic moiety and is generally assessed by measuring the 'area under the concentration time curve' (AUC), the peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}). The extent of the systemic availability is determined by the extent of drug absorbed from the site of administration. For a drug that obeys linear pharmacokinetics, the AUC and C_{max} values increase proportionately with the dose. Consequently, if two formulations / dosage form of the same drug exhibit comparative AUC values, they are considered to have similar systemic availability. The bioavailability of an oral dosage form or a drug is generally compared with an intravenous solution (100% standard), to determine the absolute bioavailability.

➢ COMPARATIVE BIOAVAILABILITY: A UNIVERSAL APPROACH:

Most bioavailability studies, whether for a new or generic product, possess a common theme. A test is conducted to identify the quantitative nature of a specific product comparison. This comparison for a new drug may be, for example, to assess the performance of an oral formulation relative to that of an intravenous dose, or perhaps the performance of a modified-release formulation in comparison to a conventional capsule. For a generic product, it is typically a comparison of a competitive formulation with a reference product.

Such commonality surrounding comparative bioavailability studies suggests a universal experimental approach



Cinitapride **Figure 1:** Illustration of the key metrics in a comparative bioavailability trial showing, for example, Test and Reference products. The maximum concentration (Cmax) occurs at the Tmax. The AUCt is the total area under the concentration versus time profile to the last sampling time. The area to ccomputing the metrics, conclusions need to be reached regarding the comparison. Statistical methods are applied to test if the metrics are sufficiently similar to be considered equivalent. When the metrics are deemed equivalent, the drug concentration profiles are regarded as fundamentally the same. To achieve this equivalence, the study products' geometric mean ratios (eg. AUC test/AUC reference), as well as their projected 90% confidence intervals for the population mean ratio, must be located within an 80 to 125% window. For the maximum concentration

(Cmax) some regulatory agencies consider it adequate if only the mean ratios are within the interval

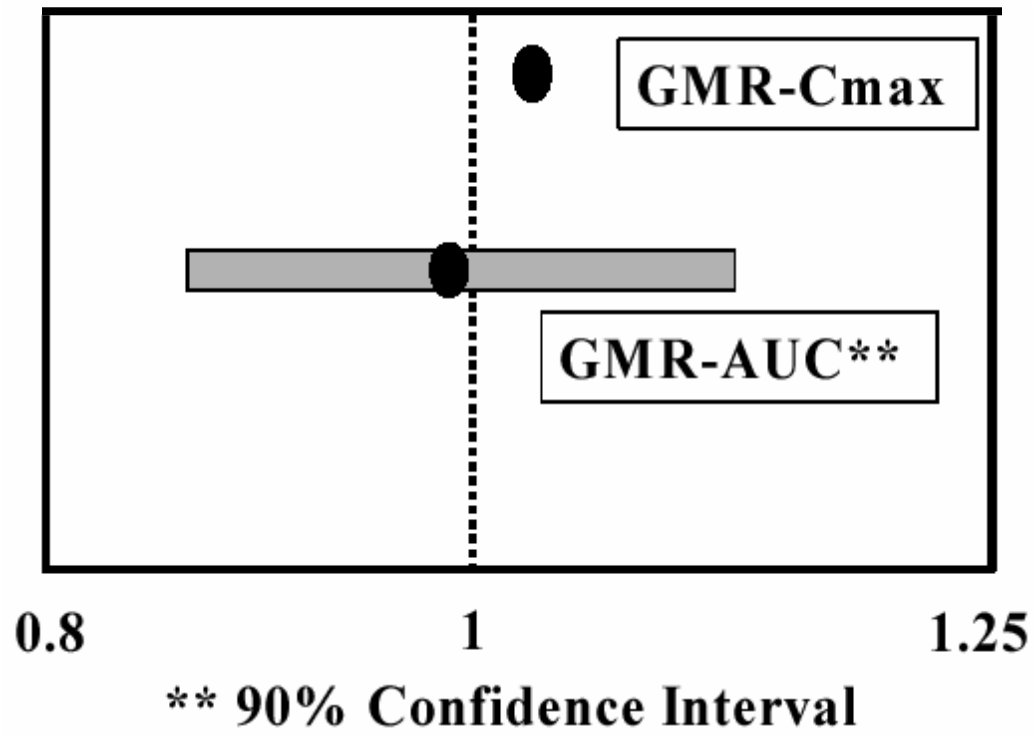


Figure 2: An illustration of the statistical criteria to be satisfied to gain equivalence status in a comparative bioavailability assessment. For example, in a bioequivalence trial, the geometric mean ratio for the test/reference Cmax (GMR Cmax) must be located between 0.8 and 1.25. The GMR AUC's (whether AUCt or AUC∞) and their computed 90% confidence intervals must reside completely within the 0.8 to 1.25.

- **BIOEQUIVALENCE:**

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. 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.

The present day bioequivalence studies are too complicated, expensive and difficult to be carried out. Two of the reasons for this difficulty are the need for many healthy volunteers and withdrawing 10-20 blood samples from an indwelling catheter from each volunteer spanning over a long period of time. Same procedure has to be repeated after a washout period, substituting the reference and test samples in the volunteers. All the samples have to be chemically analyzed and the collected data subjected to elaborate statistical analysis. The parameter 'area under the curve' can have nearly the same values for vastly bioinequivalent products as it reflects only the total amount of drug reaching the systemic circulation.

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,
- When significant changes are made in the manufacture of the marketed formulation and
- 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. Bio-equivalence cannot be claimed based on in-vitro testing only or on the basis of animal studies only. Bio-equivalence of human drugs must be determined in humans via established measures of bioavailability. By the same token animal drugs must be tested for bio-equivalence in the animal species for which the drug in intended.

Once bio-equivalence has been established via bioavailability testing in a statistically significant manner subsequent batches of the same product are deemed bio-equivalent based on in-vitro measures such as drug dissolution.

There is no such thing as increased bio-equivalence. The statement of increased bio-equivalence makes no sense. A product can be either bio-equivalent or bio-inequivalent A product can't be more bio-equivalent or less bio-equivalent.

- **Pharmacokinetic Measurement:**

Direct (e.g., rate constant, rate profile) and indirect (e.g., C_{max}, T_{max}, mean absorption time, mean residence time, C_{max} normalized to AUC) pharmacokinetic measurements are limited in their ability to assess rate of absorption. From these direct or indirect measurements of absorption rate to measures of systemic exposure. Cmax and AUC can continue to be used as measures for product quality BA and BE, but more in terms of their capacity to assess exposure than their capacity to reflect rate and extent of absorption.

- **Before Peak concentration:**

For orally administered immediate-release drug products, BE may generally be demonstrated by measurements of peak and total concentration. An early concentration measure may be indicated on the basis of appropriate clinical efficacy/safety trials and/or pharmacokinetic / pharmacodynamic studies that call for better control of drug absorption into the systemic circulation (e.g., to ensure rapid onset of an analgesic effect or to avoid an excessive hypotensive action of an antihypertensive). In this recommends use of partial AUC as a Before Peak concentration. The partial area should be truncated at the population median of T_{max} values for the reference formulation. At least two quantifiable samples should be collected before the expected peak time to allow adequate estimation of the partial area.

- **Peak concentration:**

Peak concentration should be assessed by measuring the peak drug concentration (C_{max}) obtained directly from the data without interpolation.

- **Total concentration:**

For single-dose studies, the measurement of total concentration should be: Area under the plasma/serum/blood concentration-time curve from time zero to time t (AUC_{0-t}), where t is the last time point with measurable concentration for individual formulation. Area under the plasma/serum/blood concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$), where $AUC_{0-\infty} = AUC_{0-t} + Ct/z$, Ct is the last measurable drug concentration and z is the terminal or elimination rate constant calculated according to an appropriate method. The terminal half-life ($t_{1/2}$) of the drug should also be reported.

The following pharmacokinetic parameters are required for submission:

- Plasma concentrations and time points
- Subject, period, sequence, treatment

- AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, z , and $t_{1/2.}$
- Intersubject, intrasubject, and/or total variability, if available
- C_{min} (concentration at the end of a dosing interval),
- C_{av} (average concentration during a dosing interval),
- Degree of fluctuation [$(C_{max}-C_{min})/C_{av}$], and
- Swing [(Cmax- C_{min})/C_{min}] if steady-state studies are employed.

The following statistical information required for AUC_{0-t}, $AUC_{0-\infty}$, and Cmax:

- Geometric mean
- Arithmetic mean
- Ratio of means
- Confidence intervals

Logarithmic transformation should be provided for measures used for BE demonstration.

Rounding off of confidence interval values:

Confidence interval (CI) values should not be rounded off; therefore, to pass a CI limit of 80-125, the value should be at least 80.00 and not more than 125.00.

➢ 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.

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 (18-55 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's 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.

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):**

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

- **Two-sequence and four-period design:**

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

- **Four-sequence and four-period 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**

	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 1 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

- **Crossover design for four medications (Williams' design):**

	Period			
Sequence	1	2	3	4
1	R	T3	T1	T2
2	T1	R	T2	T3
3	T2	T1	T3	R
4	T3	T2	R	T1

- **Wash out period:**

Subsequent treatments should be separated by periods long enough to eliminate the previous dose before the next one (wash-out period). Sampling should be done long enough to cover at least 80% of the area under the plasma concentration curve as extrapolated to infinity. The extrapolation should be based on knowledge of the dominating elimination half-life.

- **Subjects:**

The number of subjects required is determined by the error variance associated with the primary characteristic to be studied (as estimated from a pilot experiment, from previous studies or from published data), by the significance level desired, and by the deviation from the reference product compatible with bioequivalence and with safety and efficacy. It should be calculated by appropriate methods and should not be smaller than 12. The deviation allowable usually is 20%. The number of recruited subjects should always be justified.

Bioavailability studies generally will be performed with healthy volunteers. If feasible, they should belong to both sexes and be between 18 and 55 years old to minimize intra- and inter-individual variation subjects should be standardized as much as possible and acceptable. They should preferably be fasting at least during the night before administration of the products or they should take a standard meal at a specified time before the treatment. Time and preferably composition of meals taken after the treatment should be standardized. Because fluid intake may profoundly influence gastric passage, it should be strictly standardized and specified. The subjects should not take other medicines during a suitable period before and during the study. They should preferably abstain from food and drinks, which may interact with circulatory, gastro-

intestinal, liver or renal function (e.g. alcoholic or xanthine-containing beverages). Preferably they should be non-smokers. If smokers are included they should be identified as such. In some cases (e.g. study of high clearance substances) even posture or physical activity may have to be standardized.

- **Reference and test product:**

All investigated products must have been prepared in accordance with GMP-rules. Batch control results of the test product should be reported. Generic products, being pharmaceutical equivalents or alternatives are normally compared with the corresponding form of a well-established "Innovator" medicinal product (reference product). The applicant should justify the choice of reference product. The test product will mostly originate from a test batch. After scale-up samples of the product the production batches should be compared with those of the test batch, and they should show the same dissolution rate "in vitro" in a discriminatory test. The study sponsor will have to retain a sufficient number of product samples for the accepted shelf life plus one year to allow repetition of "in vitro" and "in vivo" studies at the request of the authority.

Data analysis:

The aim of a bioequivalence study is to demonstrate equivalence within the acceptance range regarded as clinically relevant. The primary concern in bioequivalence assessment is to limit the risk of erroneously accepting bioequivalence. Only statistical procedures, which do not exceed the nominal risk of 5%, can be approved, and among them the one with the smallest risk of erroneously rejecting bioequivalence should be selected.

In case of a parametric approach the inclusion of the classical 90% confidence interval for the chosen measure of relative bioavailability within the acceptance range (bioequivalence range) is the procedure of choice. This procedure is equivalent to the rejection of two one sided hypotheses concerning bioinequivalence at the nominal 5%-level. According to present views concentrations and concentration-related characteristics (e.g. AUC, MRT) should preferably be tested after logarithmic transformation. If the assumption of a lognormal (AUC, Cmax) distribution or normal (t_{max}) distribution in the parametric approach is doubtful, a corresponding non-

parametric approach is recommended. This approach may also be chosen as the general statistical approach to evaluate all bioavailability characteristics throughout a given study. Assumptions on the design or statistical analysis should be discussed.

- **"In vitro" Dissolution**

The results of "in vitro" dissolution tests, obtained with the batches of test and reference products that were used in the bioavailability or bioequivalence study should always be reported. The specifications for the "in vitro" dissolution of the product should be derived from the dissolution profile of the batch that was found to be bioavailable or bioequivalent.

- **Reporting**

The report of a bioavailability or bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with GCP-rules. This implies that the signature of the study monitor attests the authenticity of the whole of the report. The responsible investigators should sign for their respective sections of the report. Names and affiliations of the responsible investigators, site of the study and period of its execution should be stated. The names and batch numbers of the products used in the study as well as the composition(s) of the test product(s) should be given. In addition the applicant may submit a signed statement, confirming the identity of the test product with the product, which is submitted for registration. All results should be presented in a clear way. The way of calculating the characteristics used (e.g. AUC) from the raw data should be specified. Deletion of data should be justified. If results are calculated using pharmacokinetic models the model and the computing procedure used should be justified. Individual plasma concentration/time curves should be drawn on a linear/linear, and facultatively also on a lin/log scale. All individual data and results should be given, also of eventually dropped-out subjects. Dropout and withdrawal of subjects should be reported and accounted for. A representative number of chromatograms should be included. The analytical validation report should be reported.

- **Absolute bioavailability:**

It is the fraction effectively absorbed after extravascular administration of a 'Drug', when compared to the administration of the same 'Drug' intravenously, which has, by definition, a 100%bioavailability.

- **Adverse Event:**

An adverse event is any untoward medical occurrence in clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment.

- **Analysis Of Variance (ANOVA):**

ANOVA 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 (Treatment), subject and period; hence it is a 3-way ANOVA.

- **Area under the curve (AUC):**

Area under the curve is the total area under the biological fluid (serum, blood, etc.) concentration-time curve as determined by the Trapezoidal rule.

- **Bioavailability studies:**

It involves the determination of 'Drug' concentration in the blood or urine. Concern with how quickly and how much of a 'Drug' appears in the blood after a specific dose is administered.

- **C_{max}:**

This is the maximum 'Drug' concentration achieved in systemic circulation following 'Drug' administration.

- **Case Report Form:**

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

- **Essentially similar products:**

"A proprietary medicinal product will be regarded as essentially similar to another product if it has the same qualitative and quantitative composition in terms of active principles (substances), and the pharmaceutical form is the same and, where necessary, bioequivalence with the first product has been demonstrated by appropriate

bioavailability studies carried out." Pharmaceutical products essentially similar to an "innovator" product are usually designated as "generics" or "branded generics".

- **Good Clinical Practice:**

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.

- **Informed Consent:**

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 are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed, and dated informed consent form.

- **Investigational Product:**

A pharmaceutical form of an active ingredient being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.

- **Pharmaceutical alternatives:**

Medicinal products are pharmaceutical alternatives if they contain the same therapeutic moiety but differ in chemical form of that moiety or in the dosage form or strength. The therapeutic moiety may be used in the form of salts, esters, etc.

- **Pharmaceutical equivalents:**

Medicinal products are pharmaceutical equivalents if they contain the same amount of the same active substance(s) in the same dosage forms that meet the same or comparable standards. Pharmaceutical equivalence does not necessarily imply

bioequivalence as differences in the excipients and/or the manufacturing process can lead to faster or slower dissolution and/or absorption.

- **Protocol:**

A document that describes the objective(s), design, methodology, statistical consideration, and organization of a trial. The protocol usually also give the background and rationale for the trial, but these could be provided in other protocol referenced documents. Throughout the ICH-GCP Guidance, the term protocol refers to protocol and protocol amendments.

- **Quality Assurance:**

All those planned and systematic action that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with GCP and the applicable regulatory requirements(s).

- **Quality Control:**

The operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial related activities have been fulfilled.

- **Relative bioavailability:**

Bioequivalence between 'Drug's, administered by the same extra vascular route, may be evaluated comparing pharmacokinetic parameters related to bioavailability, i.e., to the quantity absorbed and to the rate of the absorption process. Two products may be compared with one of them being considered as the reference and another is test.

- **Sponsor-Investigator:**

An individual who both initiate and conducts, alone or with others, a clinical trial and under whose immediate direction the investigational product is administered to, dispensed to, or used by a subject. The term does not include any person other than an individual (*e.g.* it does not include a corporation or an agency). The obligations of a sponsor-investigator include both those a sponsor and those of an investigator.

- **Standard Operating Procedures:**

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

- **Steady state:**

An equilibrium state where the rate of the 'Drug' input is equal to the rate of elimination during a given dose interval.

- **Suprabioavailability:**

If the new product displays a bioavailability appreciably larger than the approved product.

- **T_{max}:**

It is the time required to achieve maximum 'Drug' concentration in systemic circulation.

- **Therapeutic Equivalents:**

A medicinal product is therapeutically equivalent with another product if it contains the same active substance or therapeutic moiety and, when administered to the same individual, shows the same efficacy and toxicity as that product, whose efficacy and safety has been established.

- **Validation of analytical method:**

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method or process meet the requirements for the intended application.

2. LITERATURE REVIEW

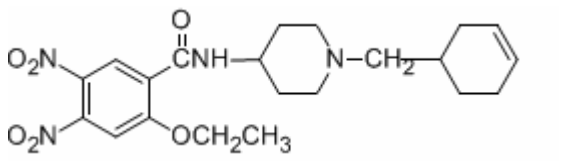
2. LITERATURE REVIEW

A General Introduction on Topic

2.1. **Drug Profile :**

Cintapro contains Cinitapride hydrogen tartrate which is a new prokinetic agent. It is a substituted Benzamide with 5-HT_2-receptor antagonist and 5-HT_4-receptor agonist activity. It is chemically described as 4-Amino-N-[3-(Cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ehoxy-5-nitrobenzamide.Its molecular formula is **$C_{21}H_{30}N_4H_4O_6$** with a molecular weight of 402.49 g/mol

2.2.**Structure of Cinitapride**:



Systematic (IUPAC) name	
4-Amino-N-[3-(Cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ehoxy-5-nitrobenzamide	
Chemical data	
Formula	$C_{21}H_{30}N_4H_4O_6$
Mol. Mass	402.49 g/mol
Pharmacokinetic data	
Bioavailability	86%
Protein binding	Less than 36%
Half life	5 to 7 hours
Excretion	Renal (circa 70%)

2.3.Abstract:-

A rapid, sensitive and specific method to quantify Cinitrapride in human plasma using risperidone as the internal standard is described. Sample preparation involved simple solide phase extraction procedure. the extraction was analyzed by high performance liquide chromatography coupled to electrospray tandem mass spectrometry API-400 (LC-MS/MS).Chromatography was performed isocratically on thermo hypurity C18 analytical column.(50mm×4.6mm.5µm i.d).the assay of cinitapride was linear calibration curve over the range 20.118 pg /ml to 2011.797 pg/ml.plasma concentration of Cinitrapride were determined by LC-MS/MS with a limit of quantification of 20.118 pg/ml that allowed an appropriate characterization of the pharmacokinetic profile of cinitapride at the therapeutic dose. the method was successfully applied to the bioequivalence tablet (1.0 mg)administered as a single oral dose.

2.4.Mechanism:-

Cinitapride,chemically 4-amino-N-[3-(Cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ehoxy-5-nitrobenzamide has the molecular formula $\mathbf{C_{21}H_{30}N_4H_4O_6}$ and molecular weight 402.49 g/mol.cinitapride is a drug that has against action to therotoninergic 5-HT2 and D2 dopaminergic receptors that has been indicated in the gastroesophageal reflux and in the functional disorder of gastrointestinal motility treatment. The therapeutic effect of Cinitapride lies on the capacity of increasing lower esophageal sphineter tone and has activity, which strong gastrokinetic activity, which generates significant increases in the gastric emptiness: besides, through the serotoninergic system it stimulates the intestinal activity. the use of Cinitapride is efficient and safe in treatment of patients with disorders in the gastric emptiness related to gastroesophageal reflux and functional dyspepsia sent irritable bowel as well as in individuals that present irritable bowel syndrome .with constipation and abdominal pain., for determination polarographic and LC-MS/MS method has been used. here we present a fast, sensitive and selective method for measuring plasma Cinitapride using LC-MS/MS with positive ion electrospray ionization using respridone as the internal standard. this method was employed in a bio-equivalence study to compare the rate and extend of absorption of cinitapride 1 mg tablets under fasting conditions in 12 healthy,adult,male subjects in a randomized crossover study.

2.5. Side Effects of Cinitapride:-

- Headache
- Upset stomach
- Vomiting
- Depression
- Cough
- Rash
- Stomach pain

2.6. Drug Interaction:-

Anticholinergic agents like atropine, scopolamine etc.may reduce the action of cinitapride. Cinitapride can enhance the effect of medication that are used for the treatment of illnesses of the nervous system and for insomnia.Cinitapride can also alter the absorption of some medicines e.g. digoxin as it stimulates gastric emptying.

Use in pregnancy, lactation and children:-

The safety of this product in pregnant women has not been established.Cintapro should only be used in pregnant women or women suspected of being pregnant only if the expected therapeutic benefits are evaluated to outweigh the possible risk of treatment . Treatment with Cintapro should be avoided during breast – feeding .the safety of this product has not been established in children.

Over dose:-

There have as yet been no reports of overdose in humans. The symptoms of overdose include drowsiness, confusion and extrapyramidal effects.Cinitapride hydrogen tartrate does not cause QT prolongation. In case of excessive overdose, the usual measures of gastric lavage and symptomatic therapy should be applied .the extra pyramidal effects should be treated with antiparkinsonians,anticholinergics or antihistaminics with anticholinergic properties.

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Objective:

i) To evaluate the comparative oral bioavailability of Cinitapride hydrogen tartarate 3 mg tablet (each containing Cinitapride hydrogen tartarate 3mg) with that of reference product cintapro 3 mg tablet (each containing Cinitapride 3 mg) of in healthy, adult, male, human subjects under fasting conditions.

ii) To assess the safety of the subjects.

3.2. Test Formulation (T):

Cinitapride hydrogen tartarate 3 mg tablet (each containing Cinitapride 3 mg)

Manufactured by: Zydus Cadila healthcare Ltd., Ahmedabad, India.

3.3. Reference Formulation (R):

Cintapro 3 mg tablet (each containing cinitapride 3 mg)

Manufactured by: Parexel institute of clinical pharmacology, Berlin,Germany.

3.4. Study Design:-

- **No. of Subjects:**

  12 + 2 (standby)

- **Blinding**

  -It was an open label study. Study Monitors and subjects involved in the study were not blinded, as it was an open label study.

  -Analysts concerned were kept blinded. The randomization schedule was in the custody of Principal Investigator and the drug dispensing raw data record was under lock and key until the completion of analysis.

  -The randomization was balanced and the code was kept under controlled access.

  -The randomization schedule was under the custody of principal investigator.

- **Design :-**

An open label, randomized, two-treatment, two sequence, two period, two way crossover, single dose bioequivalence study of Cinitapride3 mg tablet (each containing Cinitapride 3mg) manufactured by comparing with cinapro3 mg tablet (each containing Cinitapride3 mg) in healthy, adult, male, human subjects under fasting conditions.

The study flowchart, event schedule and schedule for blood sampling, drug dosing, safety assessment and meal for period I and II are given in Appendix - I, II and IV respectively.

3.5.Dose :-

- One 3mg tablet of test with 240ml water……..period(I)
- One 3mg tablet of Reference with 240ml water… .period(II)

3.6. Reference Product

Cintapro 3 mg is qualified as acceptable reference product by WHO.

3.7. Randomizations

The order of receiving test and reference product for each subject during the two periods of the study will be determined according to randomization schedule (generated using SAS® version 9.1.3).

The randomization will be balanced and the code will be kept under controlled access.

Principal investigator will have the custody of original randomization schedule. The analyst in the laboratory will not have the access to randomization code, till the end of statistical analysis. In case the standby subjects are to be analyzed, the analyst will analyze only those subjects as informed by the principal investigator, so that randomization sequence is balanced.

The person involved in the dispensing of study drugs will be accountable for ensuring compliance to randomization schedule.

3.8. Sample Schedule:-

- **16 blood sample will be collected… …….period(I)**

Predose(0.0h),0.25,0.50,0.75,1.00,1.25,1.50,2.00,
3.00,4.00,6.00,8.00,12.00,16. 00, 20.00, 24.00 hours.

- **16 blood sample will be collected … …… …..period(II)**

Predose(0.0h),0.25,0.50,0.75,1.00,1.25,1.50,2.00,
3.00,4.00,6.00,8.00,12.00,16.00,20.00,24.00 hours.

3.9. Diet and water :-

a) At least 10-14 hours prior to dosing
b) A standardized diet of approximately 2600 - 2800 calories per day will be provided to the study subjects on the day of dosing. (Refer Appendix - V)
c) Subjects will be instructed to abstain from alcohol and xanthine containing food and beverages (chocolates, tea, coffee or cola drinks), cigarettes and tobacco products, for at least 48 hours, prior to dosing, for each period, and during their participation in the study.

3.1.0. Washout Period: - There will be washout period of at least 7 days from the Completion of dosing between two consecutive periods.

3.1.1. Sampling Procedure:-

Samples will be collected through an indwelling cannula placed in a forearm vein. The pre-dose samples will be collected within one hour prior to drug dosing. The post-dose samples will be collected within 2 minutes of the scheduled time where the end time of collection to the nearest minute would be recorded. Any deviations above two minutes would be recorded as protocol deviation.

Intravenous indwelling cannula would be kept in place as long as required by injecting not more than 0.5 ml of 5 IU/ml of heparin in normal saline solution during the collection of multiple samples. In such a case, the blood sample would be collected after discarding the first 0.5 ml of heparinised blood from the tubing. Blood may also be withdrawn from vein by using disposable syringe and needle if the cannula is blocked. The proposed time point against the clock time have been and given in Appendix - IV, 'Schedule for blood sampling, drug dosing, safety assessment and meals'.

3.1.2.Handling of Samples

Each blood sample withdrawn will be transferred into graduated polypropylene tubes containing 0.1 ml of heparin (5000 IU / 5 ml). The samples collected at each time point will be centrifuged at 10°C and 3200 rpm for 10 minutes to separate plasma, after receiving the blood samples from all the subjects. The separated plasma will be aliquoted in duplicate in prelabelled polypropylene tubes during each period. These tubes will then be transferred to a deep freezer maintained at –20±5°C for temporary storage and finally to a deep freezer maintained below –50°C or colder for storage.

The tubes will be labelled with Study Number, Period Number, Date, Subject Number, Time Point (hrs), and Aliquot No.

3.1.3. Study Termination

The principal investigator reserves the right to terminate the study at any time for safety reasons or in the best interest of the subject's welfare. The ethics committee

can also cancel the study for major ethical violations. The subjects would be briefed on the reasons for the termination and compensated adequately.

3.1.4. Subject Selection, Monitoring and Assessments:-

- **Eligibility Assessment**

For the purpose of this study, the following eligibility assessments will be carried out before enrolling any volunteer into study.

I. Screening

The screening will be carried out after taking an initial informed consent from volunteers for study screening procedures and will include the following:

1. Demographic data, including sex, height and weight, BMI, nutrition status, diet, history of smoking, substances of abuse (Benzodiazepines, Opiates and Amphetamine), alcohol, blood donation and participation in drug research study
2. Medical and treatment history including present complaints (if any), relevant past medical history, family history, history of any allergy to food or drug, medication history in the last six months
3. Complete physical examination including recording of vital signs (B.P, Pulse, Temperature and respiration) and systemic examination
4. 12-lead ECG for heart rate, rhythm and specific finding (if any)
5. Chest X-ray (PA view)
6. Blood and urine samples will not be sent for laboratory examination if the subject fails in eligibility assessment during medical examination

II. Laboratory Parameter Investigations

1. Complete blood count – erythrocyte count, platelet count, haemoglobin, PCV, leucocyte count, ESR and differential leucocyte count

2. Blood grouping (if previously not performed by Bioequivalence department of Zydus cadilaPharmaceuticals Ltd.)
3. Biochemistry – blood glucose (fasting / random), serum: sodium, potassium, chloride, calcium, TG and blood cholesterol
4. Hepatic profile – SGOT, SGPT, GGTP, Alkaline Phosphatase and serum bilirubin (Total, Direct, Indirect)
5. Renal profile – serum creatinine and serum urea
6. Urine - physical examination, chemical examination, microscopic examination and substances of abuse (benzodiazepines, opiates and amphetamine)

III. Trial Assessments :-

The following will be recorded during the conduct of the study -

- Check in and check out details
- Vital details
- Meal distribution details

-Time of dose administration

-Target and actual blood sampling times

-Concomitant therapy changes

-Adverse events.

IV. Safety Assessments :-

Recording of vital signs and clinical examination :-

- A doctor will be available within the clinical facility whenever the subjects are housed (from check-in to checkout in each period).A physician will always be available on call during the study period.
- Period (I) :-Vital signs like sitting blood pressure and radial pulse will be measured and recorded during subject check-in ,prior to administration of dose ,1.00,2.00,4.00, and 12.00 hours after administration of dose and at checkout.
- Clinical examination of all the subjects will be done at the time of check-in and at checkout and /or at termination of the study .clinical examination

may also be done at any time during the conduct of the study if the attending physician feels necessary.In case of abnormality in vital signs or not.subjects will be questioned for well being at the time of clinical examinations and recording of vital signs.

- Laboratory assessments will be at the study at the time of screening.Hematology ,renal function,hepatic function will be done at the end of the study(last sample of period II).

V. Inclusion Criteria :-

- Male subjects aged between 18 and 45 years.(both inclusive)
- Subjects weight within ± 15% of the ideal height-weight chart of life insurance corporation of India for non-medical cases ability to communicate effectively with study personnel .
- Ability to communicate effectively with study personnel.
- Willingness to adhere to the protocol requirements.
- Be able to give consent for participation in the trial.
- Normal health as determined by personal medical history ,clinical examination and laboratory examination data during screening (within the clinically acceptable range)

VI. Exclusion Criteria :-

- History of hypersensitivity to cinitapride or any of the product components .
- History or presence of cardiovascular ,respiratory ,hepatic ,renal, gastrointestinal,Endocrine,immunological,dermatological,neurologic,psychiatric disease or any other body system involvement.
- History or presence of significant alcoholism or drug abuse within the past one-year.
- History or presence of significant smoking (more than 10 cigarettes per day) or consumption of tobacco products.

- Difficult with donating blood.
- Blood pressure less than 100/60 (systolic/diastolic) mm Hg or more than 140/90 mm Hg .
- Pulse less than 55/minute or more than 110/minute .
- Febrile
- Any ECG or X-Ray abnormalities during screening.
- Major illness during 3 months before the screening period.
- Presence of clinically significant abnormal laboratory values during screening .
- Subject who have participated in drug research studies within past 3 months .
- Subject who have donated one unit (350 ml) of blood I the past 3 months .
- Subject who are found positive in alcohol breathe test and urine test for drug of abuse at the time of check in during both the periods .

VII. Withdrawal Criteria :-

The investigator may withdraw a subject from the study for any of the following-

- The subject suffers from significant intercurrent illness or undergoes surgery during the course of the study .
- Any subject found to have entered the study in violation of this protocol .
- Any subject who requires the use of an unacceptable concomitant medication .
- If it is felt in investigator's best interest to continue .
- Any subject who wishes to withdraw his consent

3.1.5. Restriction

- **Dosing**

After overnight fasting of at least of 10 hours, test or reference products will be administered at 0.00 hours to the subjects as per the randomization schedule with 240 ml of water under the supervision of the Medical Officer during each period of the study.

Compliance for dosing after drug administration will be assessed by examination of the oral cavity of the subjects by trained study personnel immediately after dose administration in each period.

However, measurement of cinitapride concentrations in the plasma will be carried out for 12 subjects, successfully completing both the periods of the study. The plasma samples of standby subjects will be analysed in case of dropouts.

3.1.6. Clinical Residency:-

The clinical phase of the study will be carried out at the clinical facility of Bioequivalence department of Zyduscadila Healthcare Ltd. Subjects will be admitted and housed in the clinical facility prior to 11.00 hours before the administration of the dose during each period of the study. In each period, subjects will be discharged after 24.00 hours post dose, if not suffering from any adverse events. In case of any adverse events, the subjects will be kept under observation until the abatement of the events.

3.1.7.Pharmacokinetic Analysis:-

Pharmacokinetic parameters of Cinitapride will be calculated using the SAS® system version 9.1.3:

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

C_{max}: Maximum measured plasma concentration following each treatment.

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

$AUC_{0-\infty}$: The area under the plasma concentration versus time curve, from zero to 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.

K_{el}: Apparent first order elimination or terminal rate constant calculated from semi log plot of the plasma concentration versus time curve. The parameters will be calculated by linear least square regression analysis using at least the last three non-zero plasma concentration.

$T_{1/2}$: Time required for the plasma drug concentration to decrease to one half.

3.1.8. Analysis of Variance (ANOVA)

The log-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$) will be analysed using an ANOVA model with the main effects of sequence, subject nested within sequence, period and 'treatment'. A separate ANOVA model will be used to analyze each of the parameters. A 5% level of significance will be used for with-in subject comparison (i.e., period, 'treatment') and between-subject comparison (i.e., sequence). Each analysis of variance will include calculation of mean square error, coefficient of variance and the associated degree of freedom.

3.1.9. 90% Confidence Intervals

A 90% confidence interval for the ratio of both the products averages (geometric means) will be calculated by first calculating the 90% confidence interval for the differences in the averages (least square means) of the log-transformed data and then taking the antilogarithms of the obtained confidence limits.

3.2.0. Bioequivalence:-

The 90 % confidence interval for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of Cinitapride will form the basis for concluding the equivalence of Cinitapride in product R and T. If the point estimate of the ratio and the confidence intervals are entirely included in the range of 80 – 125 % for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} log-transformed then the treatments will be claimed to be bioequivalent.

3.2.1. Power Calculations

For analyses using the log-transformed data, the power of the ANOVA model to detect the ratio of the two products averages being equal to 125% (or 80%) at the 5 % significance level, will be calculated.

3.2.2. Ethical Consideration and Issues:-

- **Independent Ethics Committee**

This study will be carried out as per the ICH Guidelines for Good Clinical Practice (GCP) and the principles of Declaration of Helsinki (Tokyo, October 2004). The Independent Ethics committee shall review the protocol and the informed consent form for the study and no study specific procedure will be carried out until written approval is obtained from the committee. Once the study will complete, a summary of study will be send to the IEC regarding the conduct of study and occurrence of any adverse events.

- **Informed Consent Form**

Designated personnel will inform the subjects before the initiation of the study. Subjects will be required to understand and sign and date a consent form prior to initiation of any study specific activity. A copy of the signed informed consent form will be given to the respective volunteers for their reference and one sample copy will be provided in the final report. The original signed and dated ICF will be archived at Bioequivalence department of Zyduscadila Healthcare Ltd.

- **Subject Compensation**

The subjects will be compensated for the overall inconvenience borne during the study.

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

Sr. No.	Reasons of Withdrawal from the Study	Compensation
1.	Principal Investigator / Medical Officer withdraws the subjects from the study based on medical decision.	Full payment
2.	After the initiation of the study, subject withdraws on his own free will	50% proportionate participation dues
3.	The subject is withdrawn from the study on humanitarian grounds, with the permission of the Principal Investigator / Medical Officer.	100% proportionate participation dues
4.	Subject is dropped from the study due to violation of requirements of the study by the Principal Investigator / Medical Officer after signing the Informed Consent Form but before receiving any medications	No payment
5.	Subject is withdrawn from the study by the Principal Investigator / Medical Officer because of willful misinformation on present and /or past medical	No payment

	illness/history.	

4. LIST OF APPENDICES

4.LIST OF APPENDICES

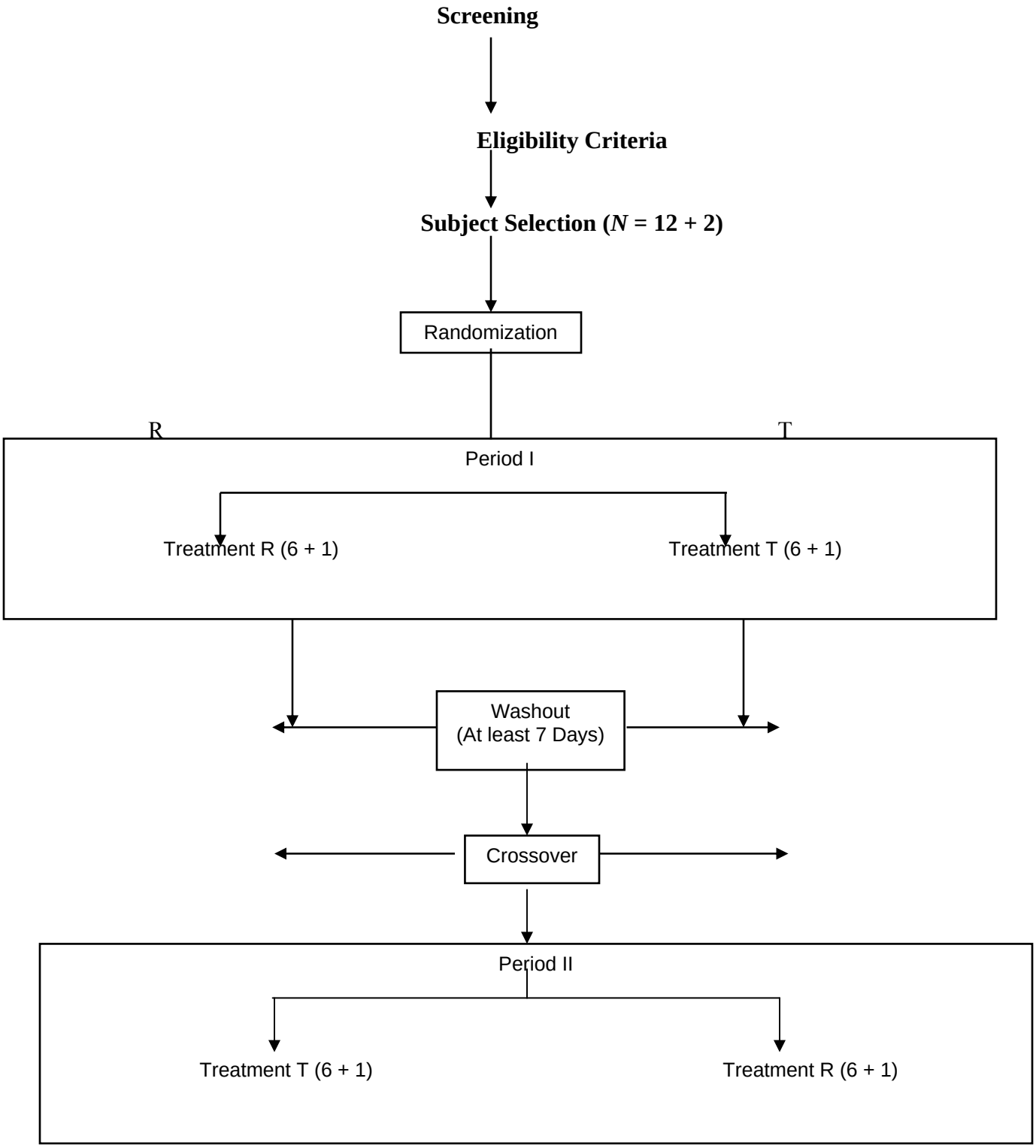
Appendix - I Study Flowchart

Appendix - II Event Schedule

Appendix - III Schedule for Blood Sampling, Drug Dosing, Safety Assessment and Meals

APPENDIX - I

1.0 STUDY FLOW CHART



***N* – Number of subjects, R – Reference Product, T – Test Product**

APPENDIX - II
EVENT SCHEDULE

Sr. No.	Requirement		Screening	Period I		Period II	
				Check-in	Check-out	Check-in	Check-out
1.	Written informed consent		*				
2.	Demographics		*				
3.	Medical and treatment history		*				
4.	Medical examination	General and systemic	*	*	*	*	*
		Vital signs	*	*	*	*	*
		12 lead ECG	*				*
		Chest X-ray PA view	*				
5.	Clinical laboratory tests	CBC (erythrocyte count, PCV, ESR, platelet count, Total and differential leucocyte count)	*				*
		LFT (SGOT, SGPT, Alk. Phosphatase and bilirubin (direct, indirect and total)	*				*
		TG, blood cholesterol	*				
		GGTP	*				
		Serum: Sodium, potassium, chloride, calcium and blood glucose (fasting/random)	*				*
		RFT (serum creatinine and serum urea)	*				*
		Urine (Routine)	*				
		Substances of abuse (Benzodiazepine, opiates and amphetamine)	*				
		Infectious diseases	*				
6.	(SQ)			*		*	
7.	Drug administration			*		*	

8.	Blood sampling		***	***

CBC: Complete blood count **LFT:** Liver function test **RFT:** Renal function test
SQ: Subject questionnaire

ECG and clinical laboratory tests will be performed to cater to the post study safety assessments at the end of period II.

APPENDIX - III

SCHEDULE FOR BLOOD SAMPLING, DRUG DOSING, SAFETY ASSESSMENT AND MEALS

2.0 DOSE ADMINISTRATION AND BLOOD SAMPLING

Time	**Dose Administration**	**Blood Sampling**															
Time Relative to Dose Administration (Hrs)	0.00	–1.00 to 0.00	0.25	0.50	0.75	1.00	1.25	1.50	2.00	3.00	4.00	6.00	8.00	12.00	16.00	20.00	24.00
Clock Time in Hrs (Days)	0800 (D 1)	0700 to 0800 (D1)	0815 (D 1)	0830 (D 1)	0845 (D 1)	0900 (D 1)	0915 (D 1)	0930 (D 1)	1000 (D 1)	1100 (D 1)	1200 (D 1)	1400 (D 1)	1600 (D 1)	2000 (D 1)	0000 (D 2)	0400 (D 2)	0800 (D 2)

3.0 SAFETY ASSESSMENT

Time	**Vitals**						**Subject Questionnaire (SQ)**		
Time Relative to Dose Administration (Hrs)	Prior to –11.00	–1.00 to 0.00	1.00	5.50	9.00	24.00	2.50	11.00	24.00
Clock Time in Hrs (Days)	Prior to 2100 (D 0)	0700 to 0800 (D 1)	0900 (D 1)	1330 (D 1)	1700 (D 1)	0800 (D 2)	1030 (D 1)	1900 (D 1)	0800 (D 2)

4.0 MEALS

Meal	**Dinner**	**Lunch**	**Snacks**	**Dinner**

Time Relative to Dose Administration (Hrs)	– 11.00	4.00	8.00	13.00
Clock Time in Hrs (Days)	2100 (D 0)	1200 (D1)	1600 (D1)	2100 (D1)

5. RESULTS AND DISCUSSION

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The individual and mean plasma Cinitapride concentrations for reference and test products are presented in Table **1** and **2** respectively. The summary statistics for untransformed and log-transformed pharmacokinetic parameters of reference and test products for Cinitapride are presented in Table 3 and 4 respectively. The comparative evaluation of various mean pharmacokinetic parameters of Cinitapride for reference and test products is presented in Table 5.

The comparative linear and semi-log plots of mean plasma Cinitapride concentration-time profiles of reference and test products are presented in Figures 1 to 2 respectively. The comparative linear and semi-log plots of individual plasma Cinitapride concentration-time profiles of reference and test products are presented in Figures 03 to 24.

- **Cinitapride :-**

  - The arithmetic mean T_{max} of the test product is 1.688 hours and that of the reference product is 1.125 hours.

  - The arithmetic mean C_{max} of the test product is 2851.06167 ng/ml and that of the reference product is 2909.05833 ng/ml.

- The arithmetic mean AUC_{0-t} of the test product is 13169.69896 ng*hr/ml and that of the reference product is 13059.02865 ng*hr/ml.

- The arithmetic mean $AUC_{0-\infty}$ of the test product is 13471.85806 ng*hr/ml and that of the reference product is 13348.27033 ng*hr/ml.

The % Bioavailability for the untransformed pharmacokinetic parameters is as follows:

C_{max} : 98.01%

AUC_{0-t} : 100.85%

$AUC_{0-\infty}$: 100.93%

The Intra C.V. % for log-transformed pharmacokinetic parameters is as follows:

C_{max} : 21.68%

AUC_{0-t} : 15.00%

$AUC_{0-\infty}$: 14.50%

The Power (%) for log-transformed pharmacokinetic parameters is as follows:

C_{max} : 62.31%

AUC_{0-t} : 90.94%

$AUC_{0-\infty}$: 92.52%

The 90% confidence intervals for log-transformed pharmacokinetic parameters are:

C_{max} : 83.21% - 114.26%

AUC_{0-t} : 90.73% - 113.14%

$AUC_{0-\infty}$: 91.11% - 112.79%

The ratio of mean AUC_{0-t} to mean $AUC_{0-\infty}$ for test is 97.76% and for reference is 97.83%.

6. CONCLUSION

6. CONCLUSION

Bioequivalence is evaluated by three pharmacokinetic parameters viz., AUC, C_{max} and T_{max} out of which AUC and C_{max} are main parameter for evaluation.

The pharmacokinetic parameters (AUC and C_{max}) of Cinitapride are within the acceptable limits of bioequivalence 80% - 125%.

Hence, it is concluded that single dose bioequivalence study of Cinitapride 3 mg tablet (each containing Cinitapride 3 mg) is bioequivalent with Cintpro 3 mg tablet (each containing Cinitapride 3 mg)

TABLE 1

Individual and Mean Plasma Cinitapride Concentrations (ng/mL) for Reference Product (R)

Sub.	Seq	0.00 Hrs	0.25 Hrs	0.50 Hrs	0.75 Hrs	1.00 Hrs	1.25 Hrs	1.50 Hrs	2.00 Hrs	3.00 Hrs	4.00 Hrs	6.00 Hrs	8.00 Hrs	12.00 Hrs	16.00 Hrs	20.00 Hrs	24.00 Hrs
1	RT	0.00	83.23	1200.74	3812.02	4036.21	3710.65	3300.19	2831.14	2172.77	1786.02	1620.60	881.15	277.04	116.77	65.83	BLQ
2	RT	0.00	0.00	479.46	3164.28	3009.00	2755.60	2541.75	1793.72	1748.48	1091.16	834.51	523.32	282.95	138.00	91.55	60.42
3	TR	0.00	275.64	1254.27	2325.25	2585.93	2297.58	2181.84	1640.96	1481.42	1276.48	869.15	516.01	220.55	112.37	70.84	50.13
4	TR	0.00	728.31	1512.44	1519.17	1232.12	1171.34	1094.65	1048.21	811.04	621.34	280.72	195.32	95.27	61.55	50.72	BLQ
5	RT	0.00	238.97	1604.67	3262.41	2744.20	2282.72	2191.26	1715.07	1609.38	1312.21	639.67	336.90	135.62	73.13	BLQ	BLQ
6	TR	0.00	57.95	1495.36	3449.42	4511.30	4585.11	3825.49	3038.42	2612.26	2221.32	1206.43	766.74	306.31	131.22	76.22	59.78
7	TR	0.00	0.00	427.15	1068.45	2087.12	3032.60	3937.48	3674.47	1902.13	1814.56	937.85	557.30	202.58	97.02	66.66	55.36
8	RT	0.00	277.57	1077.66	1079.61	1196.32	1342.14	1474.34	1825.74	1190.43	916.68	465.73	288.26	125.54	71.54	BLQ	BLQ
9	TR	0.00	BLQ	407.33	619.76	855.22	1034.20	1209.06	1646.77	1608.75	1441.28	750.81	420.48	127.71	54.37	BLQ	BLQ
11	RT	0.00	335.03	2246.97	2482.35	2513.16	2397.63	2362.65	2159.49	2000.98	1512.26	871.62	554.02	226.78	122.66	68.01	50.77
12	TR	0.00	77.97	1283.86	1754.86	1665.25	1653.00	1679.64	1503.13	1029.60	1223.06	947.65	571.80	202.86	106.82	67.41	61.48
14	RT	0.00	95.94	2028.99	4077.58	3668.76	3266.46	2863.92	2406.94	2161.99	1867.44	805.83	421.89	145.27	56.17	BLQ	0.00
Mean		0.00000	197.32818	1251.57500	2384.59667	2508.71583	2460.75250	2388.5225	2107.00500	1694.1025	1423.65083	852.54750	502.76583	195.70667	95.13500	69.65500	48.27714
S.D.		0.000000	212.145339	593.128150	1172.585762	1166.82587	1078.80184	954.917675	749.979649	519.283280	448.339962	339.369411	191.923405	70.019128	30.378408	11.425382	21.773749
Minimum		0.00	0.00	407.33	619.76	855.22	1034.20	1094.65	1048.21	811.04	621.34	280.72	195.32	95.27	54.37	50.72	0.00
Maximum		0.00	728.31	2246.97	4077.58	4511.30	4585.11	3937.48	3674.47	2612.26	2221.32	1620.60	881.15	306.31	138.00	91.55	61.48
C.V.%		-	107.51	47.39	49.17	46.51	43.84	39.98	35.59	30.65	31.49	39.81	38.17	35.78	31.93	16.40	45.10

Note: Samples having concentration below 50.02 ng/mL were set to zero and reported as BLQ.

TABLE 2

Individual and Mean Plasma Cinitapride Concentrations (ng/mL) for Test Product (T)

Sub.	Seq	0.00 Hrs	0.25 Hrs	0.50 Hrs	0.75 Hrs	1.00 Hrs	1.25 Hrs	1.50 Hrs	2.00 Hrs	3.00 Hrs	4.00 Hrs	6.00 Hrs	8.00 Hrs	12.00 Hrs	16.00 Hrs	20.00 Hrs	24.00 Hrs
1	RT	0.00	227.50	1413.74	1931.12	2465.33	3169.89	4185.44	4172.16	2943.39	2200.20	1442.90	780.81	264.95	111.10	58.83	BLQ
2	RT	0.00	80.67	597.10	1378.33	2037.09	2354.20	3222.08	3253.12	2174.52	1702.92	903.40	588.43	248.93	119.78	72.07	64.32
3	TR	0.00	154.11	1755.32	2742.07	2324.74	1962.76	1694.71	1369.98	1037.02	1007.90	567.75	361.53	162.30	72.14	BLQ	BLQ
4	RT	0.00	0.00	884.98	1356.12	1432.17	1645.78	1529.21	1820.80	1440.45	1060.66	559.67	343.01	132.07	64.40	BLQ	BLQ
5	RT	0.00	0.00	BLQ	393.41	2377.69	3028.66	2396.82	1699.86	1270.44	1080.14	503.18	341.79	118.09	55.54	BLQ	BLQ
6	TR	0.00	55.30	556.53	2795.52	3989.27	3997.69	3261.73	2776.46	2449.11	1717.82	1028.35	732.78	294.73	125.61	90.46	68.96
7	TR	0.00	0.00	1336.54	2022.23	1898.41	2036.19	2136.86	1959.68	2037.27	1443.02	742.30	415.41	212.99	110.93	78.61	67.61
8	RT	0.00	638.13	1147.07	1266.31	1135.14	1130.90	1228.77	1363.00	1032.04	851.18	418.58	219.84	115.94	75.94	60.42	54.03
9	TR	0.00	305.00	811.67	1193.18	1471.79	1461.62	1404.62	1167.44	1014.83	1482.85	1004.70	653.58	167.74	67.96	BLQ	BLQ
11	RT	0.00	228.28	2043.28	4110.56	4383.50	4668.70	4041.81	3120.64	2133.37	1727.19	978.72	610.96	234.56	110.55	66.13	BLQ
12	TR	0.00	127.54	756.73	1213.11	1628.89	1777.17	1789.94	2026.85	1907.74	1748.00	1116.87	668.92	279.32	122.02	73.70	57.80
14	RT	0.00	286.88	2411.80	3506.70	3190.43	2771.94	2299.53	2226.96	2580.14	1605.54	761.81	382.29	125.85	54.56	BLQ	0.00
Mean		0.00000	175.28417	1246.79636	1992.38833	2361.20417	2500.45833	2432.62667	2246.41250	1835.02667	1468.95167	835.68583	508.27917	196.45583	90.87750	71.46000	52.12000
S.D.		0.000000	182.729471	612.786217	1091.425823	1020.394119	1065.080659	1016.17583	909.074853	663.324219	396.297044	299.270802	184.100260	67.041724	27.924913	11.009992	26.171628
Minimum		0.00	0.00	556.53	393.41	1135.14	1130.90	1228.77	1167.44	1014.83	851.18	418.58	219.84	115.94	54.56	58.83	0.00
Maximum		0.00	638.13	2411.80	4110.56	4383.50	4668.70	4185.44	4172.16	2943.39	2200.20	1442.90	780.81	294.73	125.61	90.46	68.96
C.V.%		-	104.25	49.15	54.78	43.21	42.60	41.77	40.47	36.15	26.98	35.81	36.22	34.13	30.73	15.41	50.21

Note: Samples having concentration below 50.02 ng/mL were set to zero and reported as BLQ.

TABLE 3

Summary Statistics of Untransformed Pharmacokinetic Parameters for Cinitapride

Product / Statistics	C_{max} (ng/mL)	AUC_{0-t} (ng*hr/mL)	$AUC_{0-\infty}$ (ng*hr/mL)	T_{max} (hr)	K_{el} (1/hr)	$T_{1/2}$ (hr)
Arithmetic Mean (R)	2909.05833	13059.03000	13348.26917	1.125	0.20942	3.40972
S.D.	1086.477662	4080.384295	4082.644448	0.4707	0.039520	0.588224
C.V. (%)	37.35	31.25	30.59	41.84	18.87	17.25
Geometric Mean	2714.10276	12440.40765	12744.21854	1.049	0.20622	3.36055
Arithmetic Mean (T)	2851.06167	13169.70000	13471.85833	1.688	0.21624	3.34365
S.D.	1102.527787	4022.028471	4024.843925	0.8603	0.045064	0.739516
C.V. (%)	38.67	30.54	29.88	50.98	20.84	22.12
Geometric Mean	2646.32947	12604.31562	12919.19151	1.525	0.21179	3.27209
Ratio of Geometric Means (T/R) %	97.50	101.32	101.37	-	-	-
% Bioavailability	98.01	100.85	100.93	-	-	-
Ratio (%) for Mean AUC_{0-t} to Mean $AUC_{0-\infty}$						
Reference (R)	97.83%					
Test (T)	97.76%					

TABLE 4

Summary Statistics of Log-Transformed Pharmacokinetic Parameters for Cinitapride

Product / Statistics	C_{max} (ng/mL)	AUC_{0-t} (ng*hr/Ml)	$AUC_{0-\infty}$ (ng*hr/mL)
Least Square Mean (R)	7.90621670	9.42870501	9.45283309
Least Square Mean (T)	7.88092885	9.44179446	9.46646915
ANOVA Summary (Results : P- Values)			
Sequence	0.2215	0.6051	0.6074
Period	0.5586	0.9947	0.9936
Treatment	0.7784	0.8341	0.8216
Intra C.V. %	21.68	15.00	14.50
Power %	62.31	90.94	92.52
MSE	0.045922	0.022237	0.020813
D.F.	10	10	10
90% CI for T/R Ratio			
Lower Limit (%)	83.21	90.73	91.11
Upper Limit (%)	114.26	113.14	112.79

Noste: Log-transformed data are calculated using natural log.

TABLE 5

Pharmacokinetics Parameters for Cinitapride

Sub.	Seq.	Reference Product						Tes		
		C_{max} (ng/mL)	AUC_{0-t} (ng*hr/mL)	$AUC_{0-\infty}$ (ng*hr/mL)	T_{max} (hr)	K_{el} (1/hr)	$T_{1/2}$ (hr)	C_{max} (ng/mL)	AUC_{0-t} (ng*hr/mL)	AU (ng
1	RT	4036.21	19014.99	19314.22	1.00	0.2200	3.1501	4185.44	20094.34	203
2	RT	3164.28	13445.57	13795.82	0.75	0.1725	4.0172	3253.12	15452.72	158
3	TR	2585.93	12631.35	12915.40	1.00	0.1765	3.9268	2742.07	9460.19	978
4	TR	1519.17	6356.67	6630.41	0.75	0.1853	3.7402	1820.80	9105.71	937
5	RT	3262.41	11197.74	11492.18	0.75	0.2484	2.7902	3028.66	9129.46	934
6	TR	4585.11	20070.04	20373.85	1.25	0.1968	3.5220	3997.69	17616.07	179
7	TR	3937.48	15633.57	15918.37	1.50	0.1944	3.5652	2136.86	12772.40	131
8	RT	1825.74	8172.44	8499.62	2.00	0.2187	3.1694	1363.00	7735.18	811
9	TR	1646.77	9570.91	9771.01	2.00	0.2717	2.5504	1482.85	10729.25	109
11	RT	2513.16	14445.41	14720.84	1.00	0.1843	3.7596	4668.70	17741.91	180
12	TR	1754.86	11472.16	11851.05	0.75	0.1623	4.2709	2026.85	14353.23	146
14	RT	4077.58	14697.51	14896.46	0.75	0.2823	2.4546	3506.70	13845.94	140
Mean		2909.05833	13059.03000	13348.26917	1.125	0.20942	3.40972	2851.06167	13169.70000	134
Geometric Mean		2714.10276	12440.40765	12744.21854	1.049	0.20622	3.36055	2646.32947	12604.31562	129
S.D.		1086.477662	4080.384295	4082.644448	0.4707	0.039520	0.588224	1102.527787	4022.028471	402
Minimum		1519.17	6356.67	6630.41	0.75	0.1623	2.4546	1363.00	7735.18	811
Maximum		4585.11	20070.04	20373.85	2.00	0.2823	4.2709	4668.70	20094.34	203
C.V. %		37.35	31.25	30.59	41.84	18.87	17.25	38.67	30.54	29.8

Figure-1 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =1

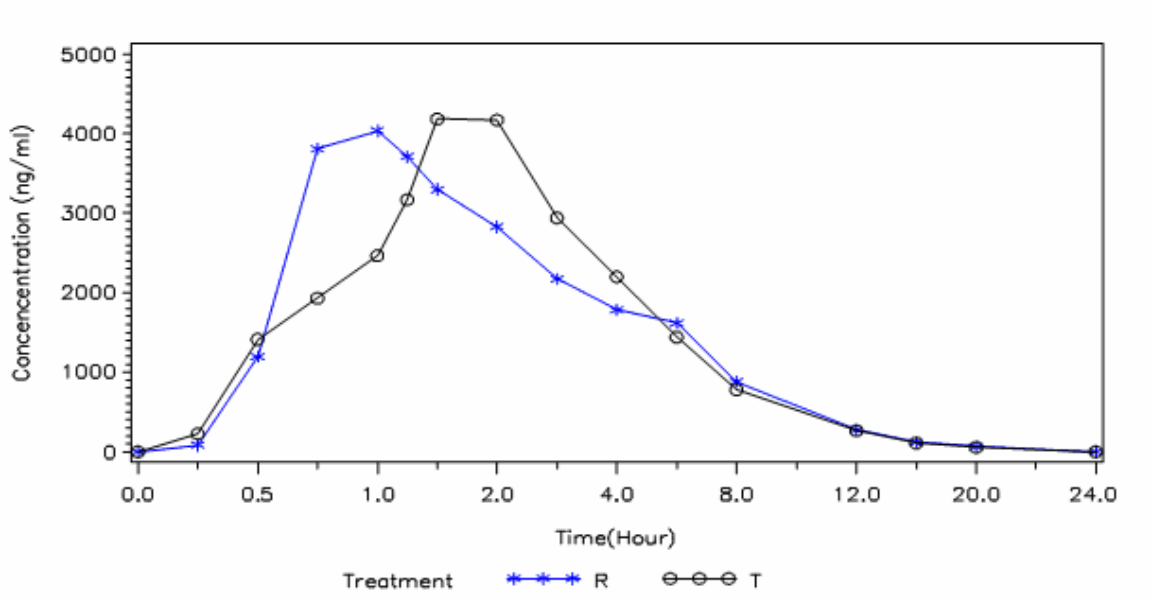


Figure-2 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =1

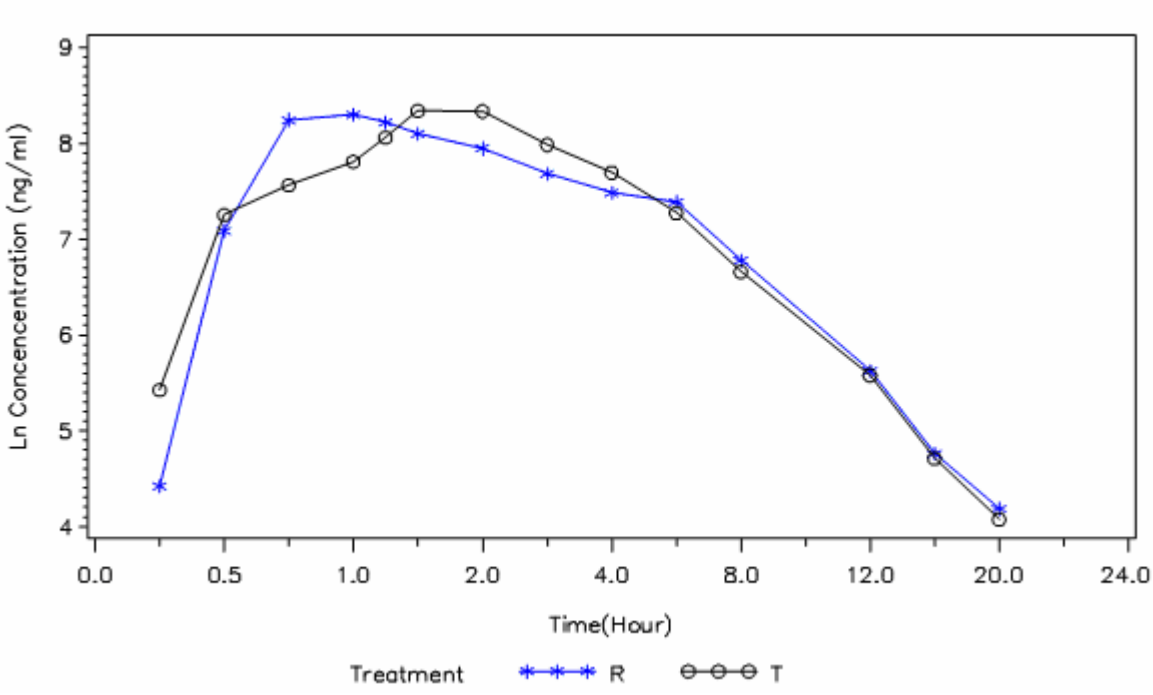


Figure-3 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =2

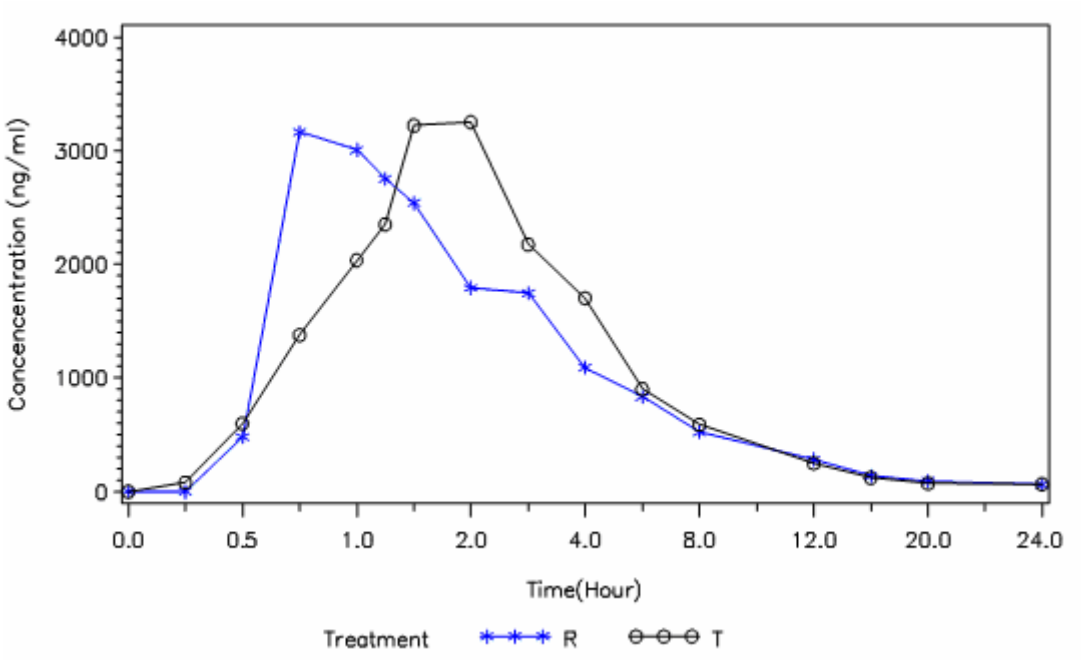


Figure-4 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =2

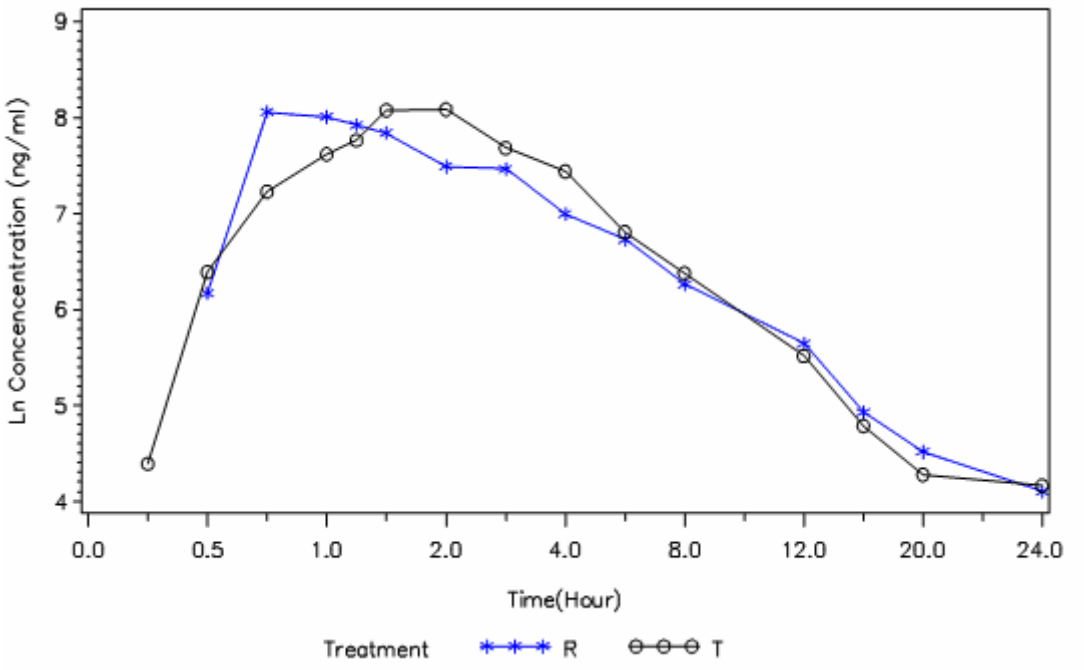


Figure-5 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =3

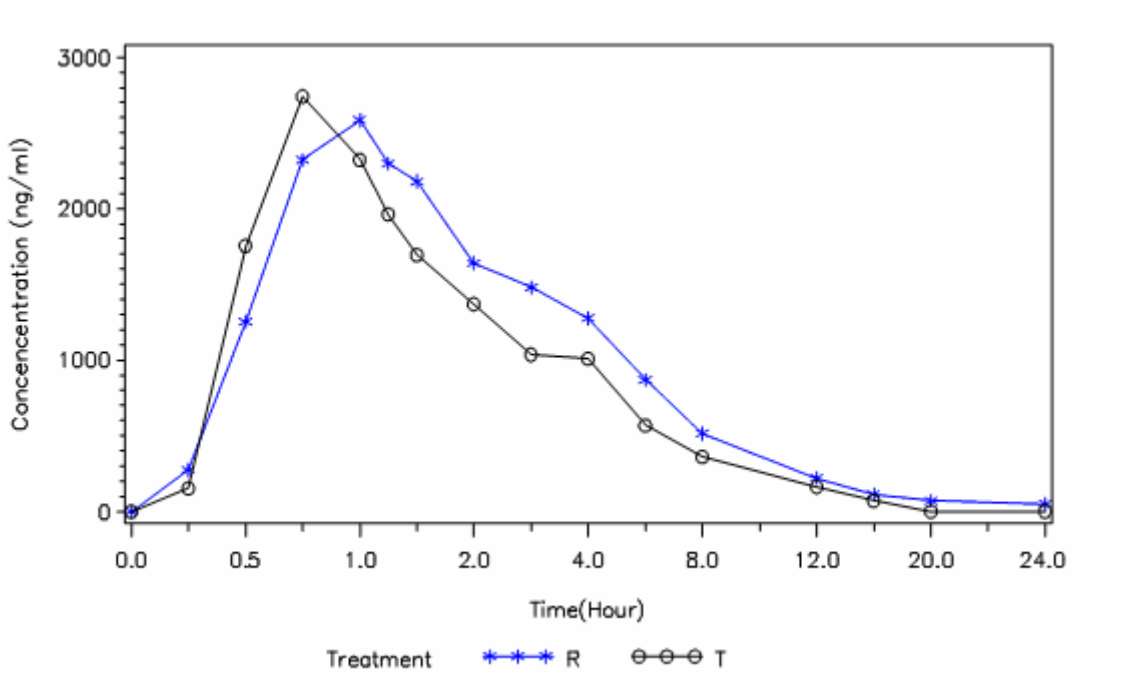


Figure-6 Comparative semi Log plot of Cinitapride Plasma Concentration (ng/ml) Vs Time . Subject No=3

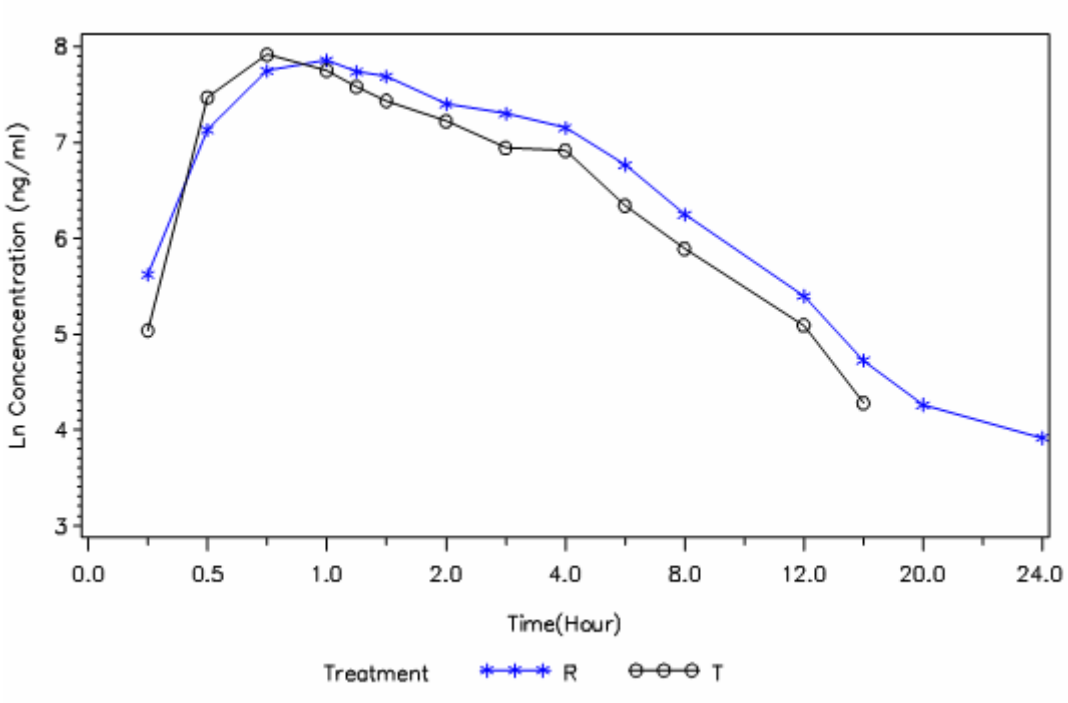


Figure-7 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =4

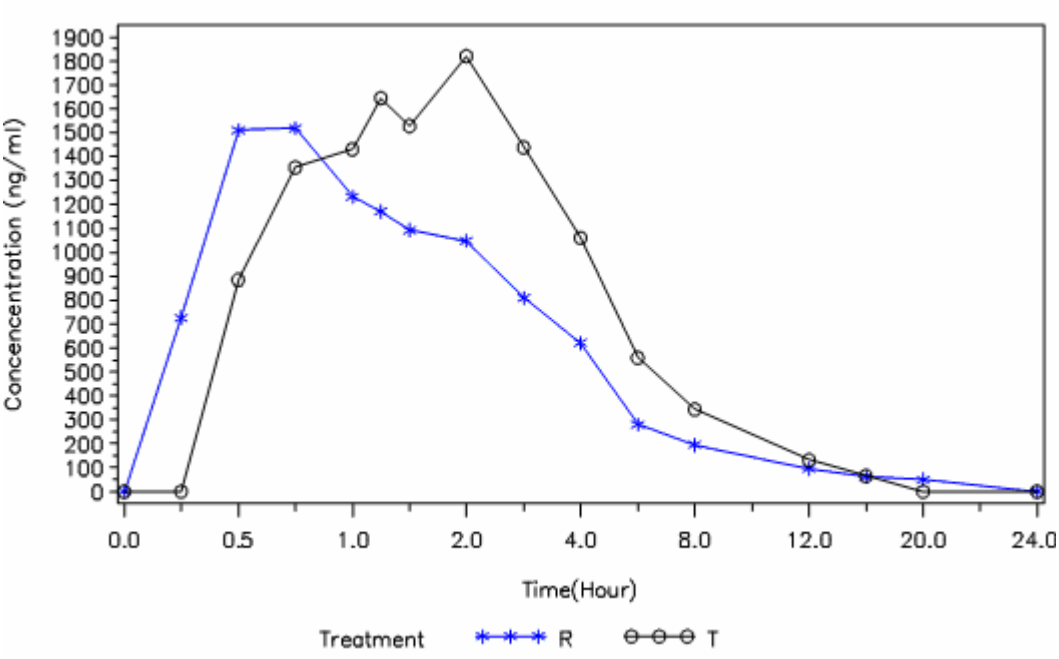


Figure-8 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =4

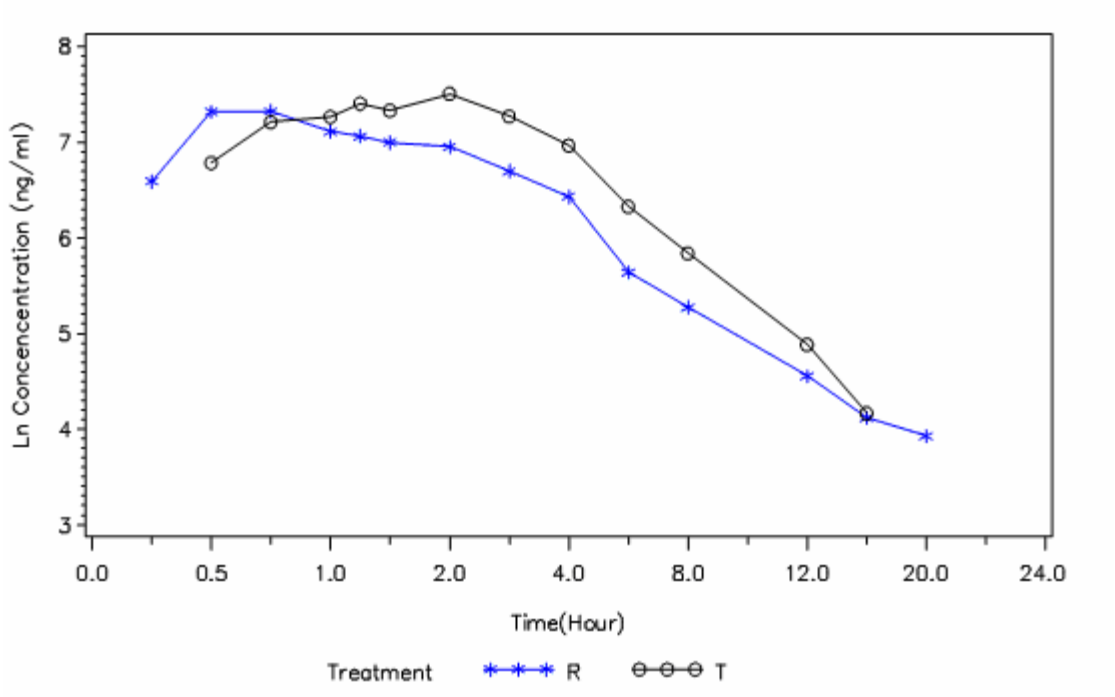


Figure-9 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =5

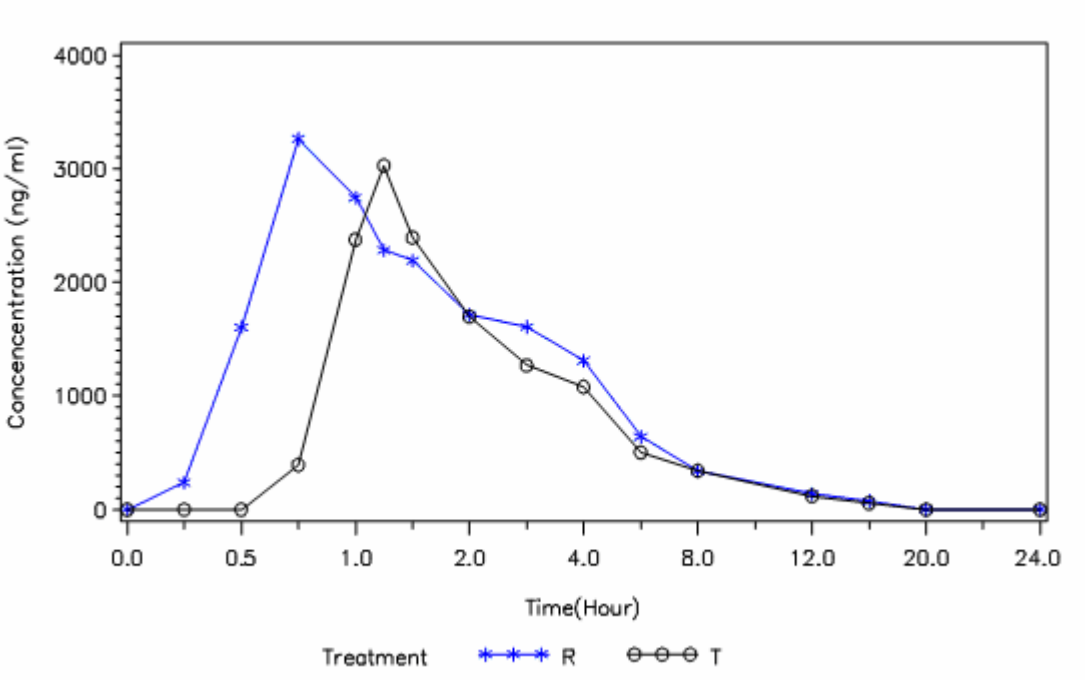


Figure-10 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =5

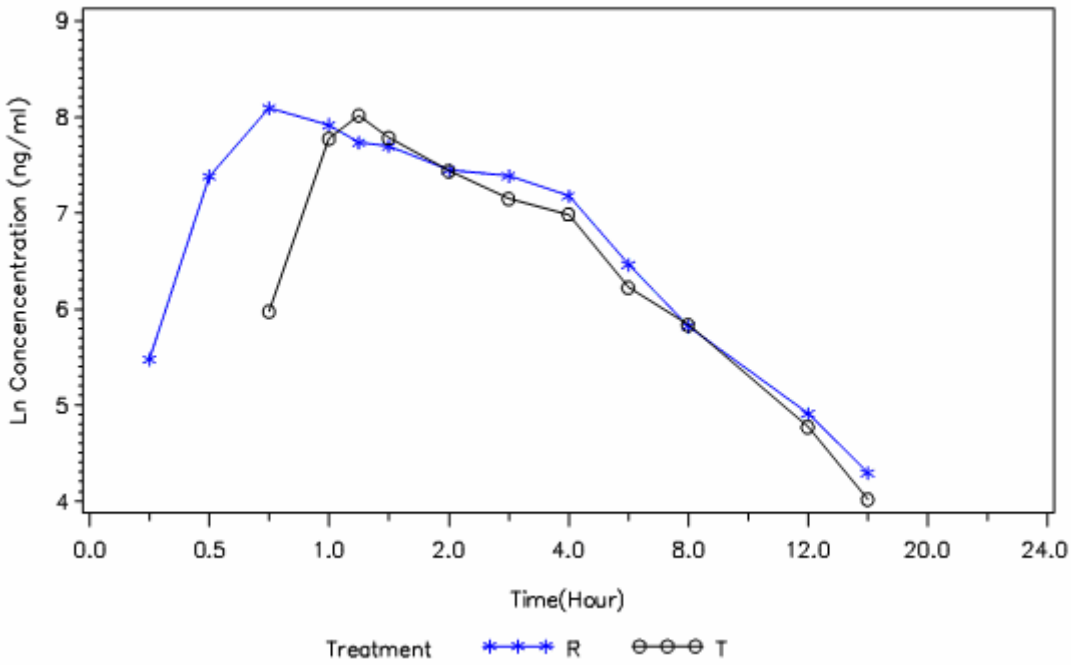


Figure-11 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =6

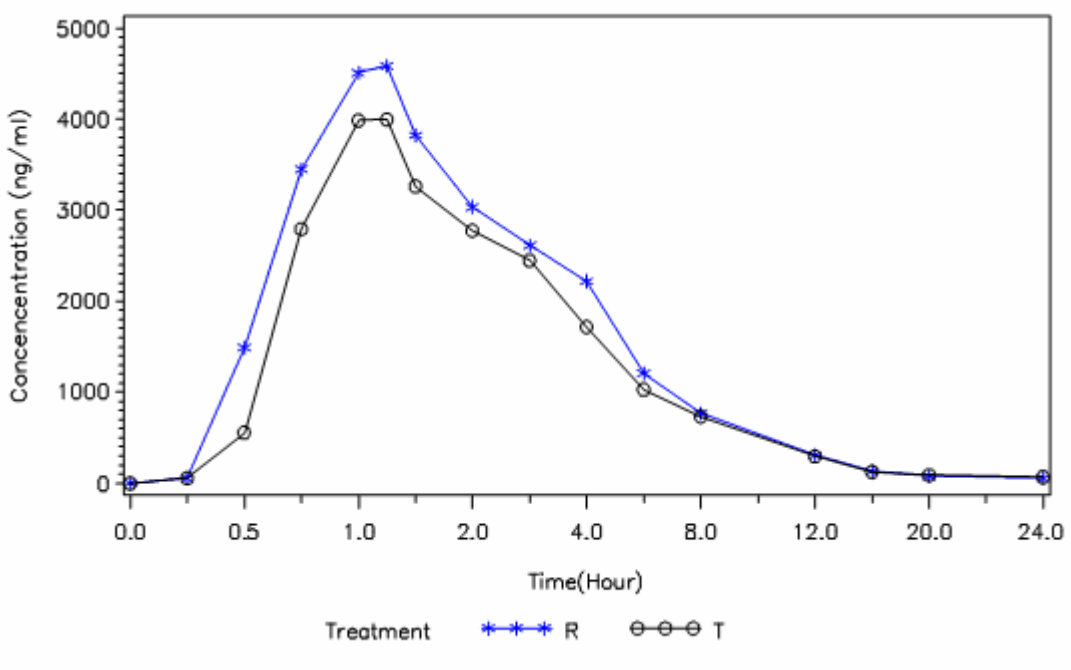


Figure-12 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =6

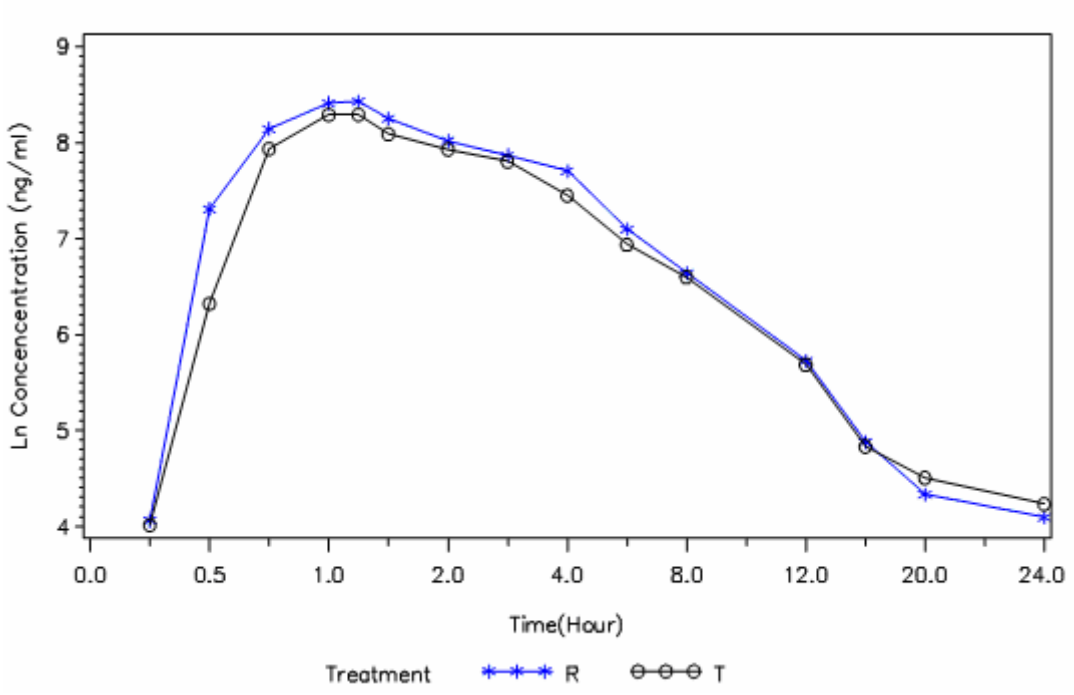


Figure-13 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =7

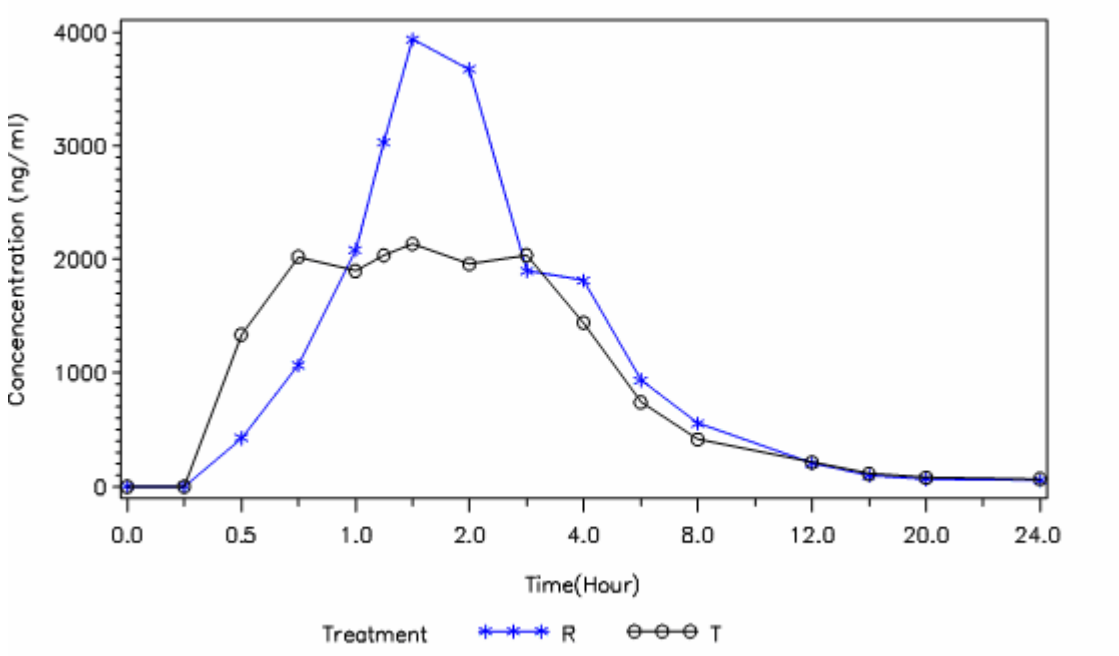


Figure-14 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =7

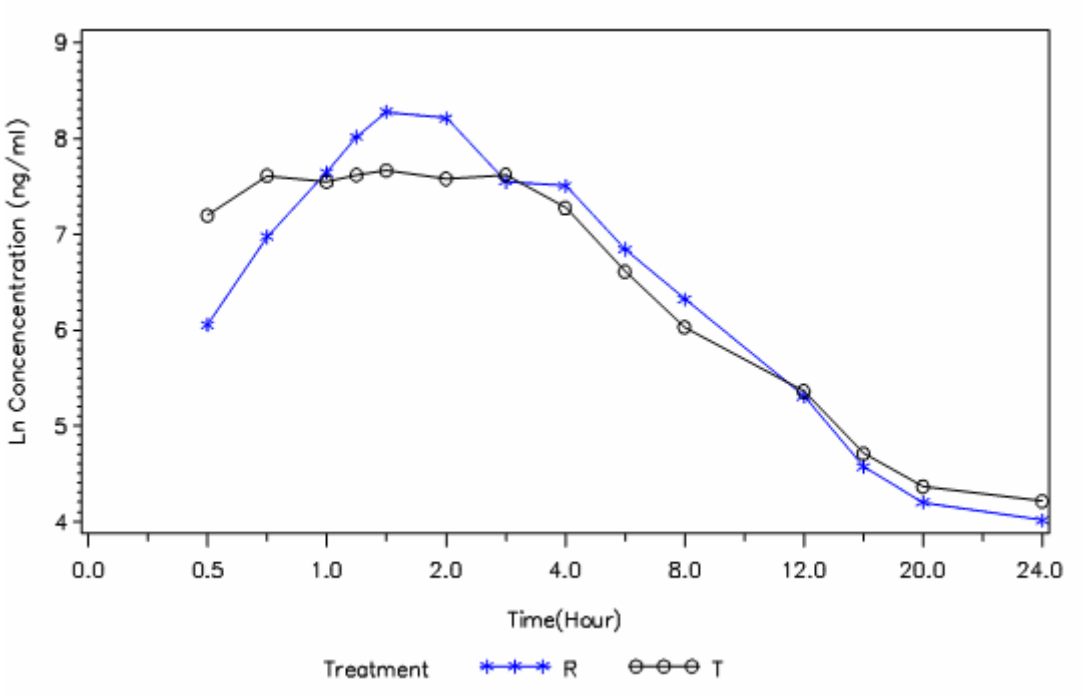


Figure-15 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =8

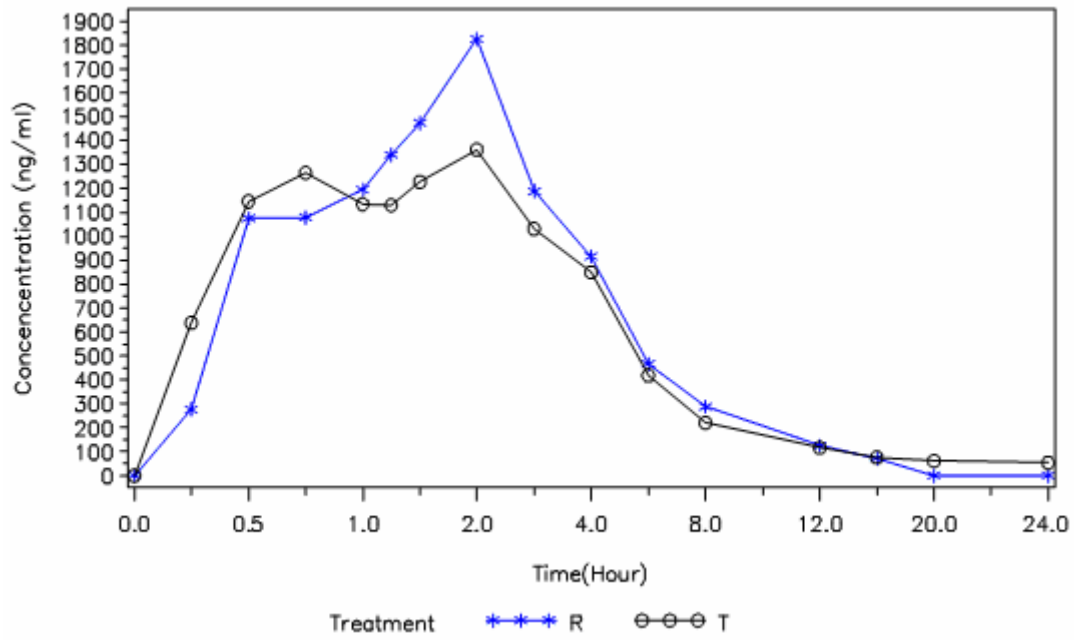


Figure-16 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =8

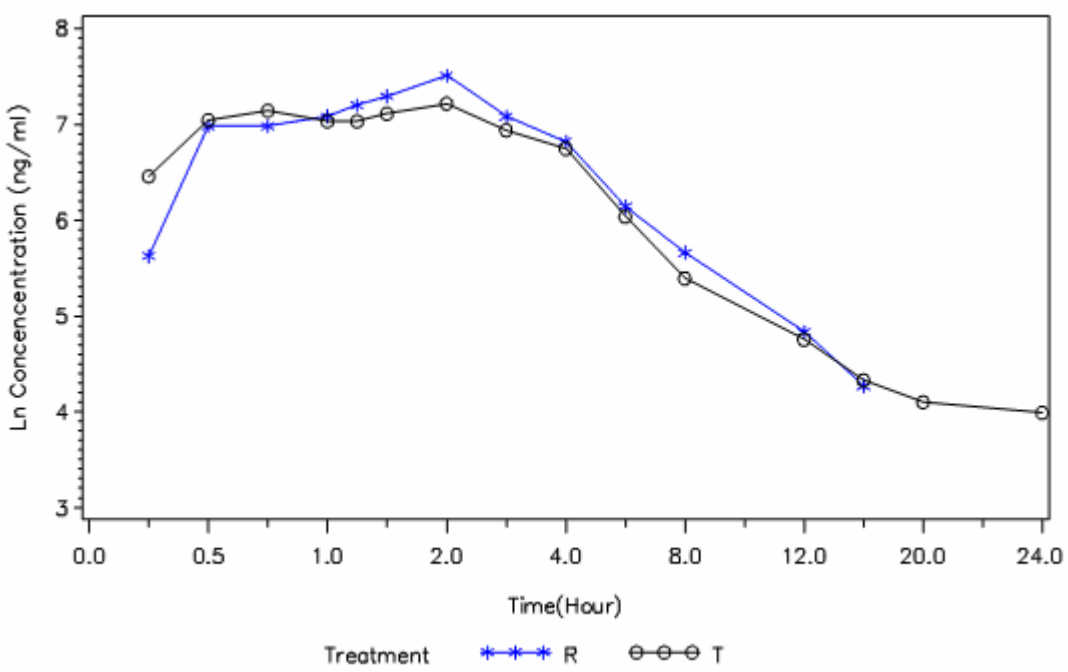


Figure-17 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =9

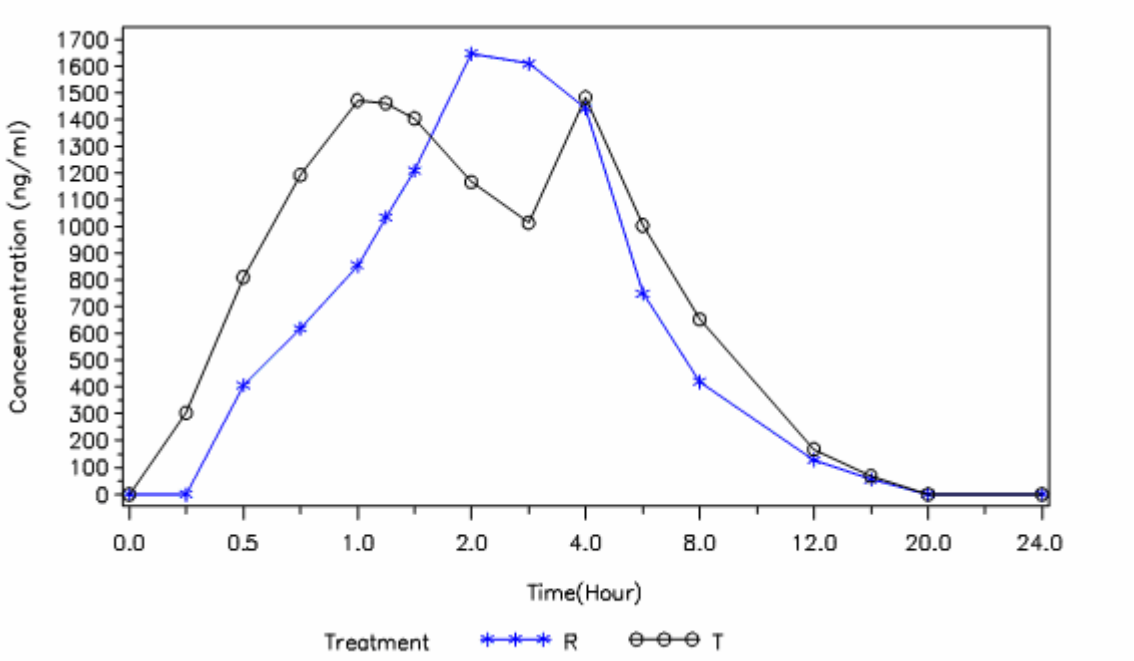


Figure-18 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =9

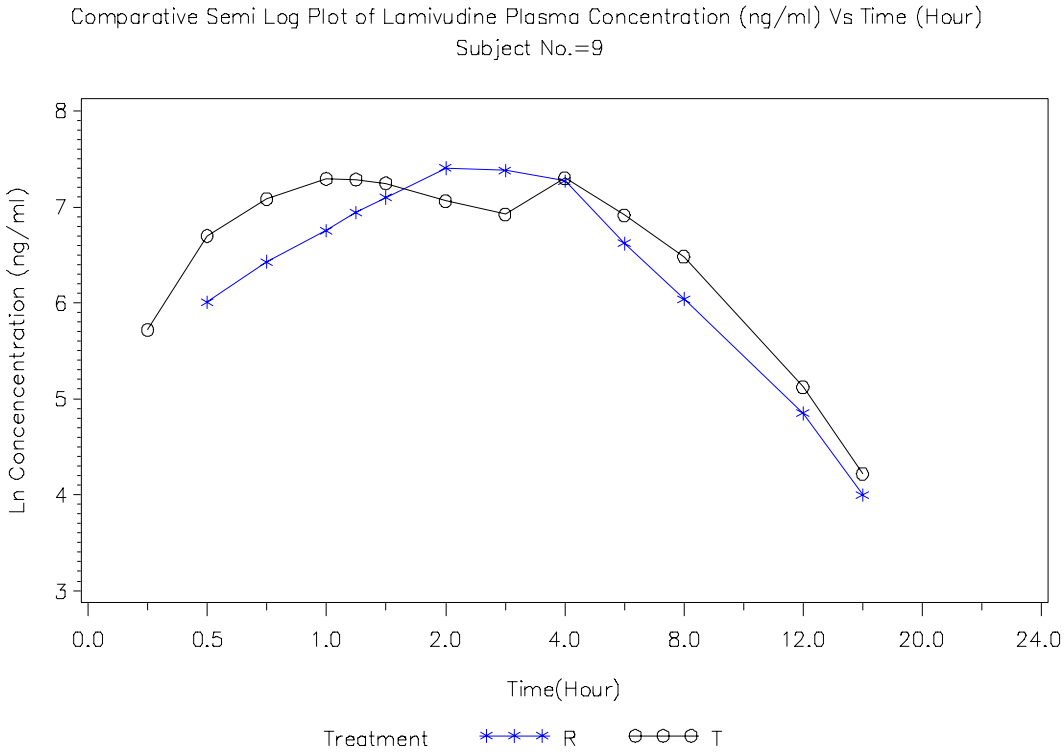


Figure-19 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =11

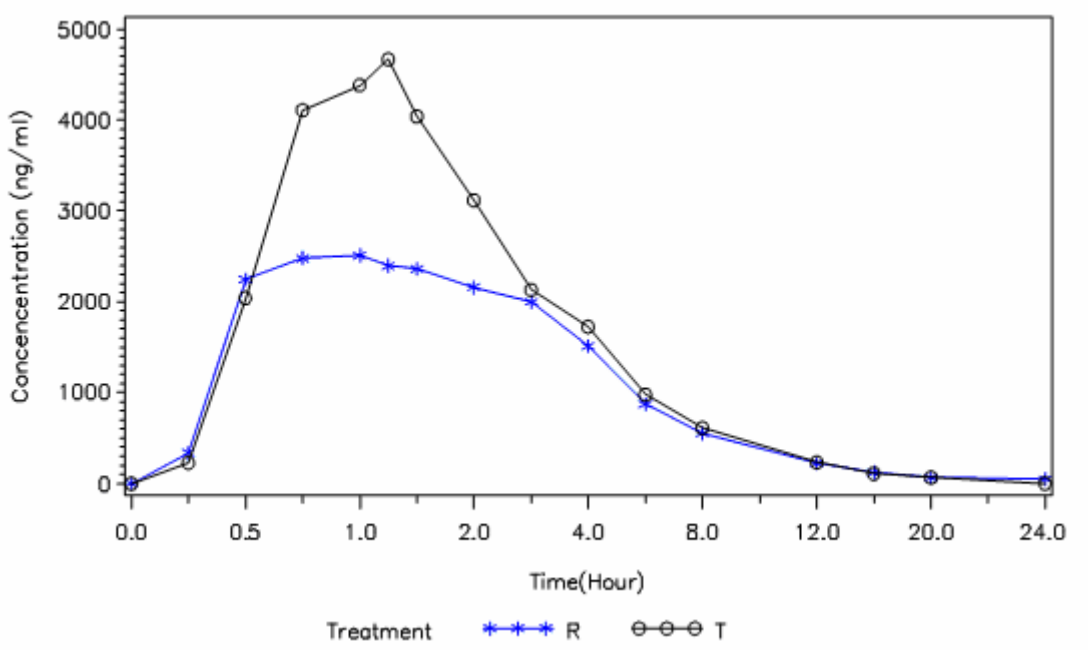


Figure-20 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =11

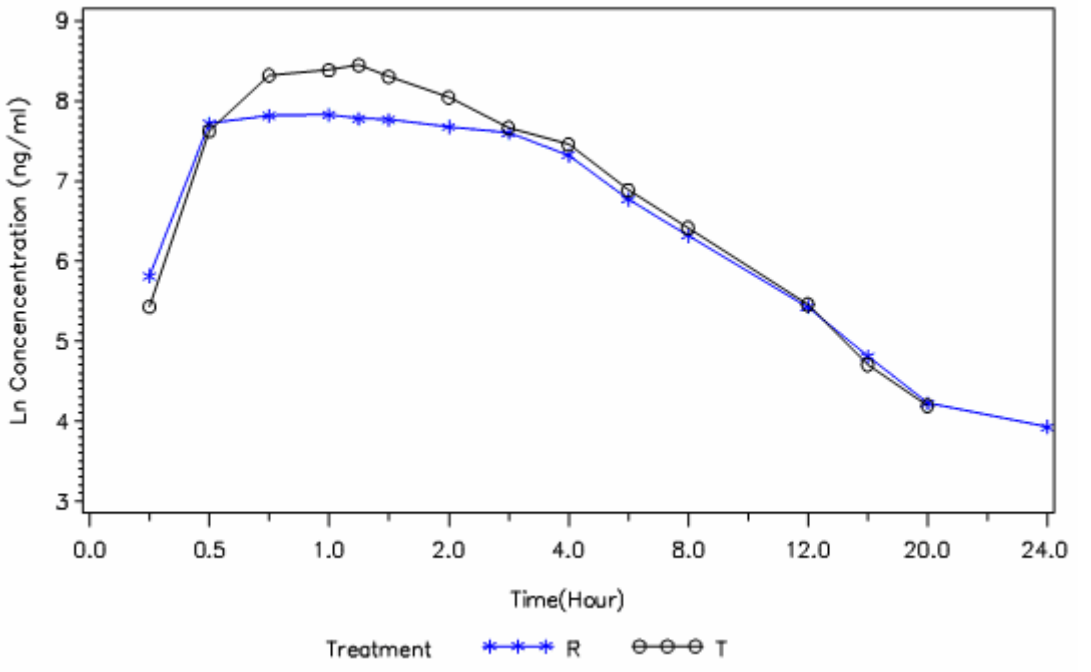


Figure-21 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =12

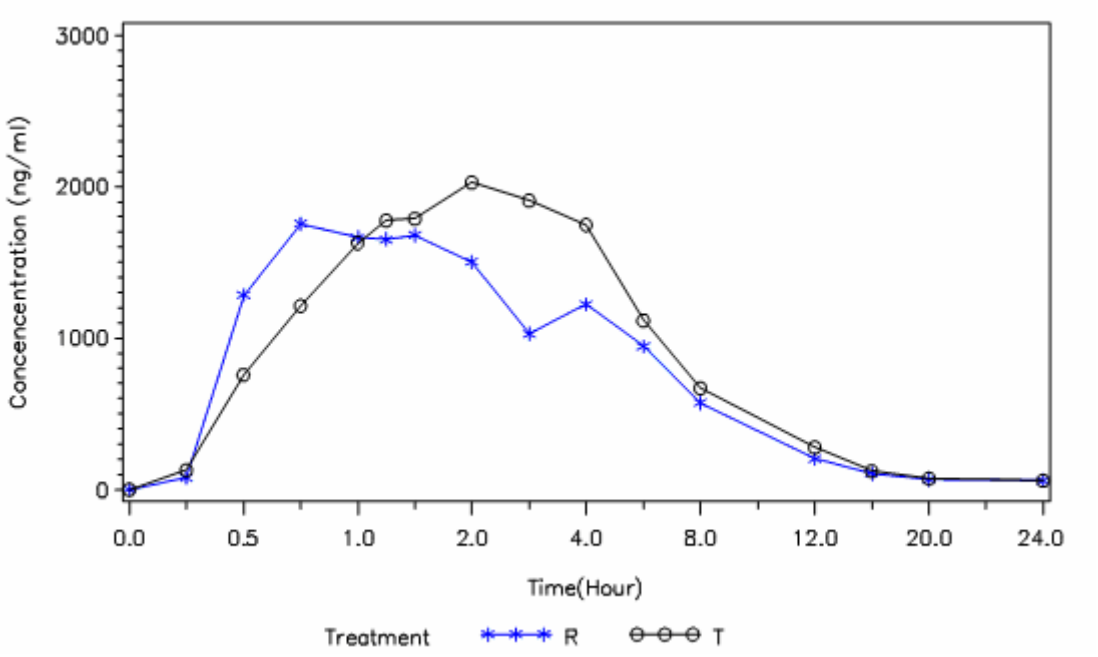


Figure-22 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =12

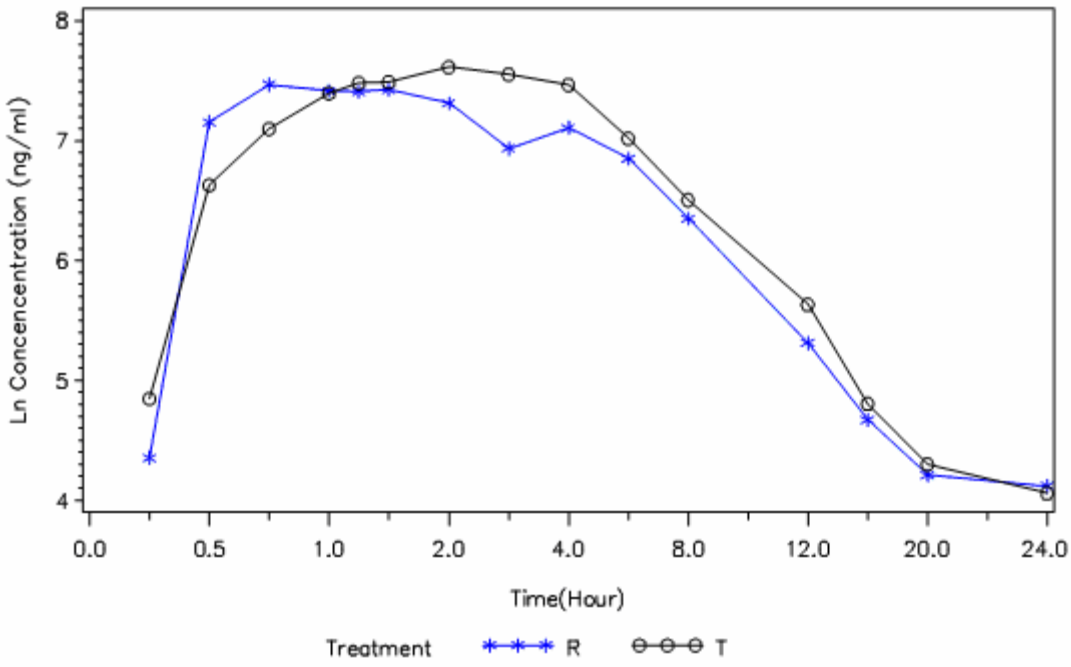


Figure-23 : Comparative Linear Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =14

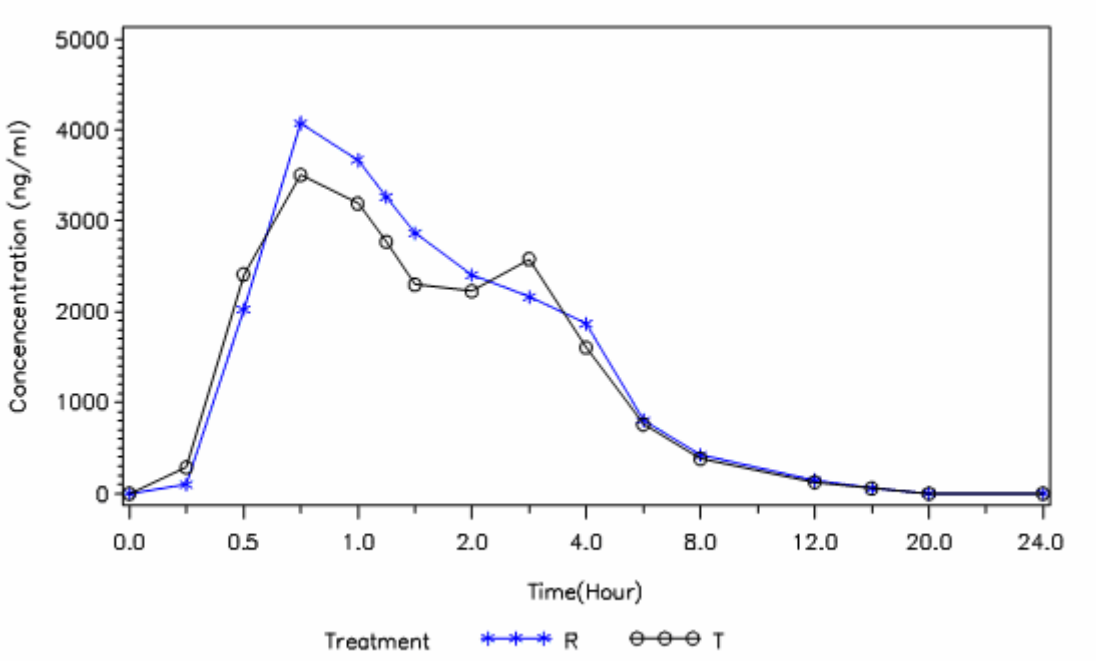
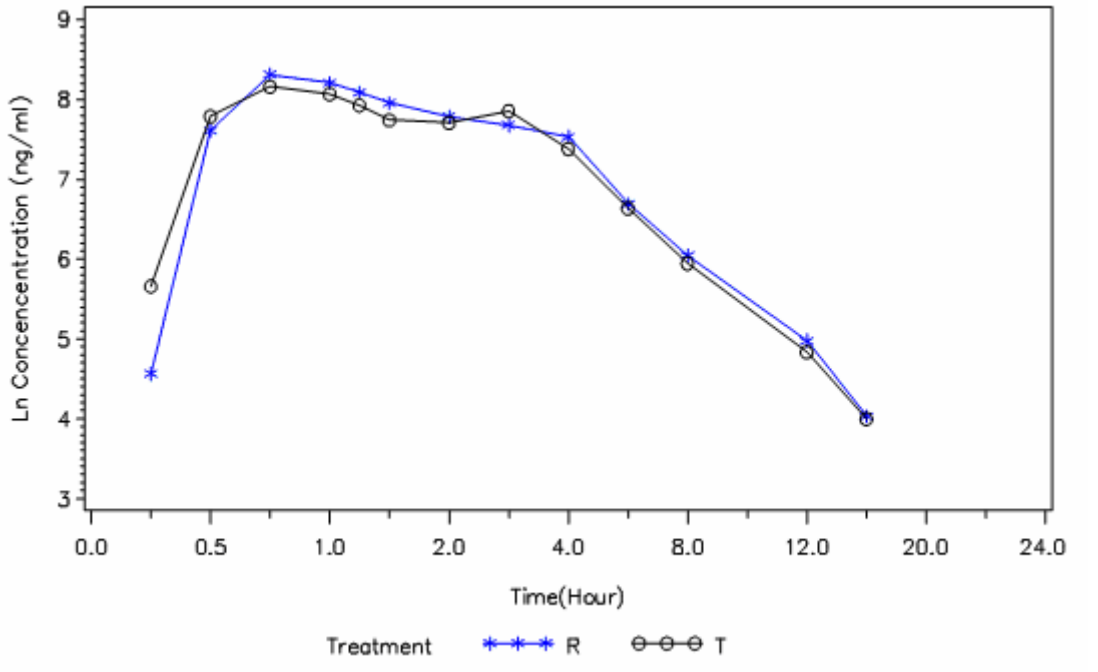


Figure-24 : Comparative Semi Log Plot of Cinitapride Plasma Concentration (ng/ml) Vs (Hours). Subject No =14



Bibliography

Bibliography

1.Article: An improved process for the preparation of Cinitapride Hydrogen Tartarate Application 341/MUM/2004 published, 2007-03-02, filed, 2004-03-19

2 Gonzalez M I, Gonzalez P C and Blanco Lopez M A, Analytica Chimica Act a 1998, 368(1), 175-181.

3 Alarcon de la Lastra C, La Casa C, Martin M J and Motilva V, Inflammation Research, 1998, 47(3),131-136.

4 Alberti J, Alvarez M, Navarro J and Salva M, Metabolismo in vitro de cinitaprida enpresencia de microsomas hepáticos humanos. Identificación del citocromo P450 responsable del etabolismo de cinitapride. Data on File, Almirall Prodesfarma S A, 2000
.
5 Crumb W J, Herg blocking profile of SD-33 (cinitapride). Data on File, Almirall Prodesfarma, S A, 2000.

6 De Graeve J, Identification by mass spectrometry of the chemical structures of in vivo human metabolites of cinitapride - Evaluation of their plasmatic and urinary elimination profiles. Data on File, Almirall Prodesfarma, 2001.

7 Fernandez A G and Roberts D J, Drugs Fut. 1991, 16 885–92.

8 Massingham R, Bou J and Roberts D J, J. Auton Pharmacol, 1985, 5, 41- 53.

9 Ames.Tonini M, De Ponti F, Di Nucci A and Crema F, Aliment Pharmacol Ther,1999, 13, 1585-1591.

10 Totah RA and Rettie A E, Clin Pharmacol Ther. 2005, 77, 341-352.

11 U. S. Food and Drug Administration. Guidance for Industry: Statistical Approaches to Establishing Bioequivalence, January 2001.

12 Department of Health Services, Food and Drug Administration.U.S., January 19,2001, 66(13),5515-5517.

13 Guidance for Industry: Bioanalytical Method Validation. U.S. Department of Health and Human Services. FDA. CDER. CVM. May 2001.

14 Marta Robert, Miquel Salva, Rosa Seggara, Marco Pavesi, Ramon Esdri, David Roberts and Georg Golor, Drug metabolism and disposition, Fast forward, April-2007.