

An Open-Label, Balanced, Randomized, Two-Treatment, Two-Period, Two-Sequence, Single-dose Crossover Pilot Oral Bioequivalence Study of Ritonavir Capsules 100 mg with NORVIR® Ritonavir Capsules Soft Gelatin 100 mg of Abbott Laboratories, USA in Healthy, Adult, Male, Human Subjects Under Fasting Conditions

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Abstract

Background: The present study was conducted to investigate the bioequivalence of Ritonavir Capsules 100 mg with that of Norvir® Ritonavir Capsules Soft Gelatin 100 mg.

Patients and methods: This study was an open-label, balanced, randomized, two-treatment, two-period, two-sequence, single dose crossover pilot oral bioequivalence study. Study was conducted in 12 healthy, adult male subjects with age ranging from 18 to 45 years. The total duration (excluding screening) of subject participation in a study was approximately 11 days including washout period of 07 days between each dosing. The estimation of Ritonavir in human plasma is carried out by using LC/MS/MS method in Bioanalytical laboratory. The pharmacokinetic parameters assessed were AUC_{0-12} , $AUC_{0-\infty}$, C_{max} , $AUC_{0-12}/AUC_{0-\infty}$, T_{max} , k_{el} and $t_{1/2}$.

Results: The geometric mean ratios (90% confidence intervals) of the test drug/reference drug for Ritonavir were 100.8 (83.64-121.51) for AUC_{0-12} , 102.1 (85.39-122.01) for $AUC_{0-\infty}$ and 97.5 (81.19-117.08) for C_{max} . The 90% confidence intervals of the test/reference AUC_{0-12} , $AUC_{0-\infty}$, C_{max} ratio of Ritonavir were within the acceptance range for bioequivalence. In this study, single dose of Ritonavir 100 mg capsule was well tolerated by both groups of subjects under fasting conditions.

Conclusion: It was concluded that the two Ritonavir capsules formulations (the test and reference products) were bioequivalent in terms of the rate and extent of absorption.

Introduction

Situations in which bioequivalence studies are required:

- 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.

Bioequivalent 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 subjects.

The purpose of the study to compare and evaluate the single-dose oral bioavailability of Ritonavir Capsules 100 mg with NORVIR® Ritonavir Capsules Soft Gelatin 100 mg of Abbott Laboratories, USA in healthy, adult, male, human subjects under fasting conditions.

General regulatory considerations for BA/BE studies

The processes of study design and workflow of BA/BE studies are presented in brief in below. The general considerations for the advancement of conducting BA/BE studies are:

- Study design and protocol.
- Bio analysis.
- Selection of appropriate analysts.
- BE metrics and data treatment.
- Statistical approaches and analysis.

Materials and methods

Design and conduct of the study

Study design

The study should be designed in such a way that the formulation effect can be distinguished from other effects. If the number of formulations to be compared is two, a two-period, two-sequence crossover design is often considered to be the design of choice.

In the present study the design followed was:

An open-label, balanced, randomized, single dose, two-treatment, two-sequence, two-period, crossover bioequivalence study.

Conduct of the study

Clinical phase

Preparation of protocol

Protocol is defined as a document signed and dated by the investigator and the sponsor that fully describes the objective(s), design, methodology, statistical considerations and organization of a study. The study protocol may also give the background and rationale for the study but these could be provided in other study protocol-referenced documents. The protocol includes all the details regarding the investigational product, the details regarding the administration of the drug, Pharmacokinetic (PK) sample withdrawal time-points, safety assessment parameters etc.

A protocol is prepared by the investigators of the study or his designee (usually a clinical pharmacologist and reviewed by various departments like analytical, statistical, QA to make necessary changes). The following chart gives an overview regarding the preparation of the protocol:

Ethical considerations

Basic principles

The study was conducted according to Good Clinical Practice, the Declaration of Helsinki, Guideline for Good Clinical Practice and all Applicable regulations and guidance.

Institutional review board

An institutional review board / independent Ethics committee reviewed this protocol and the study started only after the approval of the protocol by the institutional review board / independent Ethics committee.

Independent Ethics Committee (IEC) consists of a board of members who look into the ethical issues of the study to be conducted. The study operations can be initiated only after the protocol is approved by IEC.

An IEC should safeguard the rights, safety, and well being of all trial subjects. Special attention should be paid to trials that may include vulnerable subjects. The investigator may provide information on any aspect of the trial, but should not participate in the deliberations of the IEC or in the vote/opinion of the IEC. An IEC may invite nonmembers with expertise in special areas for assistance.

Informed consent

Designated clinical research personnel informed the subjects before initiation of the study through an oral presentation regarding the purpose, procedures to be carried out, potential Hazards and rights of the subjects during the course of the study.

ICF is designed as per the ICH-GCP and local regulatory requirements. ICF is conducted in order to get the consent from the volunteer to participate in the study. Volunteers were given all the information regarding the study including:

- Details of Investigational products
- Adverse events that may occur during the study

- The total blood loss
- The compensation to be given at the end of the study
- Regulations to be followed while participating in the study

Volunteers were given the freedom to withdraw from the study at any point of time, during the study. This consent is taken as a part of the ethical issue in conducting a BA/BE study. Every care was taken to protect the health of the volunteers. The volunteers signed on this form and gave their consent for participating in the study. Once they were enrolled in to the study, they were called 'subjects'. The enrollment in the study started with the "check-in" process.

Protocol training

After the approval of the protocol, it is discussed among the investigators of the study. The summary of the protocol that includes:

- The name of the investigational product (drug to be administered to the subjects)
- Dose to be administered
- Type of study whether it is a single center study, a fast or fed study, analyst study etc.
- Number of subjects to be enrolled in the study
- Kind of study etc, and other minute details like the Clinical Pharmacology unit (CPU) in which the subjects would be housed etc.

This summarized version of the protocol is discussed among the personnel in the facility to train them in the protocol.

Registration of volunteers

For recruiting volunteers for a study suitable volunteers are selected from the database. New people are informed and registered in the database after they gave the written consent. Generally, healthy male, adult volunteers in the age group of 18-45 years are preferred according LIC height and weight chart.

Screening

The screening was carried out after taking an initial informed consent from volunteers for study screening procedures.

Each subject was undergone a 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. The physical examination findings, ECG and the laboratory tests were considered as valid for maximum of 21 days prior to the dosing (drug administration) in first period of the study. Chest X-ray PA view will be taken within 6 months prior to dosing (drug administration) of period-1.

Clinical/laboratory diagnostic tests

| | | |
|----|----------------|---|
| 1. | Hematology: | Red blood cell count, White blood cell count, Differential white blood cell count, Hemoglobin estimation, Plat count, MCH, MCV, MCHC, RDW, Mean Plat Volume, HCT, Erythrocyte sedimentation rate (ESR), Blood grouping and RH typing. |
| 2. | Biochemistry | Serum creatinine, Blood urea, SGOT (AST), SGPT (ALT), Serum alkaline phosphatase, Total bilirubin, Blood sugar / Plasma Glucose (Random), Serum electrolytes (Sodium, Potassium and Chloride) |
| 3. | Serology | HIV (1 & 2) antibodies, HbsAg (Hepatitis B surface antigen), HCV antibodies, VDRL/ Syphilis. |
| 4. | Urine analysis | Color/Apperance, Transparency, pH, Specific gravity, Glucose, Proteins, Ketones, Bilirubin, Blood, Urobilinogen, Urine microscopic examination. |

Inclusion criteria

Subjects fulfilled all of the following criteria before including the subjects into this study:

- Healthy Male subjects aged between 18 to 45 years (inclusive of both).
- Body mass index of ≥ 18.5 kg/m² and ≤ 24.9 kg/m² and weight ≥ 50 kg.
- Healthy according to the laboratory results and physical examination, performed within 21 days prior to the commencement of the dosing in Period-1.
- Have normal ECG, Chest X-ray and vital signs.
- Subject clinical laboratory values are within normal limits or clinically insignificant as determined by physician or principal investigator to be of no clinical significance.
- Light smokers, Ex-smokers or Non-smokers (A light smoker is defined as someone smoking 10 cigarettes or less per day, Ex-smoker being defined as someone who completely stopped smoking for at least 12 months before day 1 of this study).
- Subjects able to communicate effectively and provide written informed consent.
- Subjects willing to adhere to protocol requirements as evidenced by written informed consent approved by an Independent Ethics Committee (IEC).

Exclusion criteria

Subjects were not allowed for study participation if he meets any of the following criteria:

- Any history of allergy or hypersensitivity to Ritonavir or related drugs.
- Positive test result for hepatitis B surface antigen (HBsAg), VDRL/ Syphilis, hepatitis C virus antibody (HCV Ab) or HIV-1 antibody or HIV Type 2 (HIV-2) antibody (HIV Ab).
- The study drug shall not be contraindicated for medical reasons (as stated in protocol section 3.0) to any of the study participants.
- Any history or presence of significant cardiovascular, pulmonary, hepatic, renal, gastrointestinal, endocrine, dermatological, neurological or psychiatric disease or disorder.
- History of significant alcoholism (> 3 units of alcohol per day) within one year prior to drug administration.
- History or presence drug abuse in the past one year.
- History of smoking more than 10 cigarettes per day.
- Any history or presence of cancer.
- Any history of difficulty in donating blood.
- Had clinically significant abnormal values of laboratory parameters.
- Blood pressure is $<100/60$ or $>140/90$ mmHg (Systolic blood pressure/ Diastolic blood pressure).
- Pulse rate less than 60 beats/minute and more than 100 beats/minute.
- Usage of any prescribed medication during last 14 days or OTC medicinal products, herbal products during the last 7 days preceding the first dosing.
- Any clinically significant illness during 3 months before screening.
- Participation in a drug research study/donation of blood within past 90 days.
- Consideration by the investigator, for any reason, that the subject is an unsuitable candidate to receive Study drug.

Subjects with positive for breath alcohol test, urine screen for drugs of abuse [(Cannabinoids (Marijuana / Tetra Hydro Cannabinoids-THC), Cocaine, Opiates (morphine), Amphetamine, Barbiturates, and Benzodiazepines] at the time of check-in for each period were excluded from the study.

Check-in process

The volunteers who gave their consent to participate in the study were enrolled in the study i.e. the check-in process. During this process it was checked whether the person has met all the inclusion/exclusion criteria and cleared the screening process.

They were undergone vital examination and Medical examination again to ensure they are fit for participation in the study. They would be changing into the uniforms provided to them in the facility. Once the check-in of the volunteer is completed he would be called as 'subject'. The subjects were provided with all the requirements they need including recreational activities like movies and games,

newspapers. The check in day is called as Day 0. The subjects were given standardized dinner after which they would be fasting overnight for 10 hours.

Tests performed before check-in of each period

Subject's urine was screened for drugs of abuse like cocaine, cannabinoids, amphetamines, barbiturates, benzodiazepines and opiates at the time of check-in for each period.

Alcohol breath test was performed for all subjects before check-in into each period of the study. Subject will be rejected / withdrawn from the study if the result is positive for alcohol.

Number of subjects

Total 12 healthy, adult male subjects, 18 to 45 years old volunteers were enrolled in this study.

Housing

All Subjects were checked-in into the clinical facility at 10.50 hrs before dosing on 31 Jul 2014 & 08 Aug 2014 in period-01 & period-02 respectively. They were checked-out 24 hours after dosing on 02 Aug 2014 & 10 Aug 2014 in each period period-01 & period-02 respectively.

Randomization

The test and reference products were assigned to each subject in a sequence according to a predetermined randomization schedule prepared by using SAS software 9.1.3 version. The randomization schedule prepared is as follows:

| Subject ID | Sequence | Period-01 | Period-02 |
|------------|----------|-----------|-----------|
| 1 | 1 | A | B |
| 2 | 2 | B | A |
| 3 | 1 | A | B |
| 4 | 2 | B | A |
| 5 | 2 | B | A |
| 6 | 1 | A | B |
| 7 | 2 | B | A |
| 8 | 1 | A | B |
| 9 | 1 | A | B |
| 10 | 2 | B | A |
| 11 | 2 | B | A |
| 12 | 1 | A | B |

Dispensing

As per the randomization schedule a qualified registered pharmacist dispensed the investigational products under the supervision of Quality Assurance personnel on 31 Jul 2014 & 08 Aug 2014 in period-01 & period-02 respectively. Remaining drug products were stored in their original container as retention samples. The test and reference product were stored in humidity chamber below 25°C and 60% RH \pm 5%.

The dispensed tablets were transferred to the drug-dispensing containers as unit doses. The drug-dispensing containers used for dispensing were properly labeled for the study number, period number, subject number, treatment code, initial and date of the person dispensing the product.

The details of the investigational products were as follows:

| Product | Test | Reference |
|----------------------------------|------------------------------|---|
| Treatment ID | A | B |
| Product Name | Ritonavir Capsules 100 mg | NORVIR® Ritonavir Capsules Soft Gelatin 100 mg |
| Manufactured / Distributed by | -- | Abbott Laboratories, USA |
| Strength | 100 mg | 100 mg |
| Dosage Form | Capsules | Capsules |
| Route of Administration | Oral | Oral |

Dosing of investigational product

All subjects were fasted for at least 10 hours prior to scheduled time for dosing. As per the randomization schedule, one tablet of test (A) product i.e., Ritonavir Capsule 100 mg or one tablet of reference (B) product i.e., NORVIR® Capsule Soft Gelatin 100 mg was administered to each subject with 240 mL of water at ambient temperature on 01 Aug 2014 and 09 Aug 2014 in period-01 and period-02 respectively by trained study personnel.

Subjects were instructed not to chew or crush the capsule or tablet but to consume it as a whole. Compliance for dosing was assessed by a thorough check of the oral cavity immediately after dosing. Administration of investigational products was carried out while the subjects were in sitting posture and they were instructed to remain seated for two hours after dosing in each period except when clinically indicated to change the posture or in case of any natural exigency. Thereafter, the subjects were allowed to engage in normal activities while avoiding severe physical exertion.

Diet and water

Subjects were fasted for 10 hours before dosing in each period. Drinking water was prohibited for two hours before and two hours after dosing. At other times, drinking water was provided ad libitum. Meals or snacks were provided at 4 hr (lunch), 8 hr (snacks), 12 hr (dinner) and 24 hr (check-out breakfast) after dosing in each period.

Restrictions to subjects

Smoking

All subjects were instructed to abstain from smoking for at least 24.00 hours prior to dosing till last sample collection in each period.

Medications

Subjects were asked about their medication history in the past, particularly last 14 days for usage of any prescribed medication and last 7 days for the usage of any OTC medicinal products, herbal products preceding the first dosing and were instructed not to take any medication until completion of the study.

Diet

All subjects were instructed to abstain from any xanthine-containing food or beverages (tea, coffee, chocolates, soft drinks etc.), grapefruit juice or related products and alcohol or related products for at least 24.00 hours prior to dosing till the last sample collection in each period and were prohibited from consuming above mentioned products, during their in house stay. Subjects were instructed not to consume/chew any tobacco containing products {pan masala, gutkha, supari (betel nut)} etc. from

24.00 hours prior to dosing of each period and till last sample collection in each period.

Activity

All subjects were dosed at the fixed time and were remained in sitting position for the first 2.00 hours following drug administration except while going for sampling and medical examination/vitals or clinically indicated/for natural exigency. Further subjects were ambulatory but they were advised to avoid severe physical exertion.

Drinking water

Drinking water will be prohibited for two hours before and two hours after dosing. At other times, drinking water will be provided ad libitum.

Collection of blood samples

The sampling schedule should be planned to provide an adequate estimation of C_{max} and to cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of absorption. This is generally achieved if the AUC derived from measurements is at least 80% of the AUC extrapolated to infinity. For most drugs, 18 to 30 samples, including a pre-dose sample, should be collected per subject per dose. This sampling should continue for at least three or more terminal half lives of the drug¹⁶. The exact timing for sample collection depends on the nature of the drug and the input from the administered dosage form. The sample collection should be spaced in such a way that the maximum concentration of the drug in the blood (C_{max}) and terminal elimination rate constant (λ_z) can be estimated accurately.

According to the literature T_{max} is 2 hours and $T_{1/2}$ ranged between 3 - 5 hours for Ritonavir after oral use, the below sampling schedule is decided using above T_{max} and $T_{1/2}$ values.

In this study, in each period, a total of 21 (1 x 6 mL) venous blood samples were collected from each subject as per the following schedule:

Pre-dose (0.00 hr), 0.33, 0.67, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 7.00, 8.00, 10.00, 12.00, 16.00, 24.00 and 36.00 hours post dose in each period.

Blood loss

The total blood loss combining both the periods [including 291 mL for pharmacokinetic analysis, 19 mL of discarded heparinized blood prior to each post-dose sample collected through cannula (except for pre-dose and ambulatory samples), about 10 mL blood collected for pre-study screening and about 10 mL blood collected for post safety assessment] did not exceed 291 mL.

Sampling procedure

Blood samples were collected through an indwelling cannula placed in a forearm vein. Heparin lock technique was used to prevent the clotting of the blood. At each sampling time point, blood sample was withdrawn and transferred to a sample collection tube containing K_3 EDTA as anticoagulant. Before each blood sample is collected except for pre-dose sample (0.00 hours) and ambulatory sample (36.00 hrs), 0.5 mL of heparinized blood was withdrawn and discarded to prevent heparin interference with sample analysis. For ambulatory sample collection i.e. 36.00 hrs, direct venipuncture was done.

The pre-dose blood sample was collected before dosing and the post-dose samples were collected within ± 2 minutes from the scheduled sampling time during in-house stay. For the ambulatory samples, the sampling was done within ± 60 minutes from the scheduled sampling time. During collection of blood sample at each time point the mid-point of the minute was considered to calculate the nearest minute, which was recorded on the appropriate form. The deviations greater than mentioned in the protocol from the scheduled sampling time were reported as protocol deviations.

Sample separation and storage

After collection, blood samples were placed in ice water bath till start of centrifugation, Within 30 minutes from the time of collection, the blood samples were placed in a refrigerated centrifuge and then spun at 3000 rpm for 10 minutes at 4°C. As soon as possible, the plasma obtained was separated

and transferred into two different polypropylene tubes/RIA vials. Each tube/vial will be labeled with Project No., Period No., Subject No., Sampling time point and Aliquot No. 1 mL of plasma was separated and transferred into aliquot 1 and the rest of plasma into aliquot 02.

All samples were stored at a temperature of -20°C or below for interim storage at the clinical site until transferred to analytical site.

Sample sorting

Once all the samples from the subjects were collected, the samples are sorted. The sorting was done by separating the aliquots containing samples of different time points of each subject into easy sealing bags. Various conditions are maintained while sorting the samples, like the maintenance of low temperatures. Sorting was done in presence of dry ice to prevent the exposure of samples to room temperature and also to prevent their degradation due to thawing. These bags were sealed into various boxes and stored in the deep freezer, which are later handed over to the bio-analytical department with proper documentation for further processing.

Safety monitoring

Subjects were monitored for their well being by recording vital signs. Clinical Examination was carried out and recorded at check-in, before dosing and at checkout. Vital signs (sitting blood pressure, pulse rate and oral temperature) measurement was carried out and recorded at check-in, before dosing of investigational products (in the morning of the day of dosing), at 1, 2, 4, 6, 8 (within ± 45 minutes) hours after dosing and at checkout. Clinical examination and measurement of vital signs were also being carried out at other times during the conduct of the study when the attending physician felt it necessary.

About 10 mL of blood was collected from all the subjects for safety evaluation [haematology and biochemistry] at the end of the study.

The normal vital signs range is as follows:

| | |
|------------------|------------------|
| Temperature | 97.8 °F-99 °F |
| Pulse Rate | 60-100 beats/min |
| Respiration Rate | 14-20/min |
| Systolic BP | 100-138 mm of Hg |
| Diastolic BP | 60-88 mm of Hg |

If any adverse event is observed either by clinical staff or reported by subjects at times other than scheduled times will also be recorded.

Reporting of adverse events

A total of three AE's (One AE during period 01 in-house and Two AE's during post study safety evaluation) during the study.

Subject number 08 had experienced the adverse event (Dizziness) during period 01 in-house of the study which was possibly related to the study drug and mild in intensity and resolved completely without sequel.

Post study safety evaluations of laboratory parameters were found to be within acceptable limits for all the subjects except for subject number 02 & 10. Subject number 02 was found to have (Increased total bilirubin levels) while evaluating the post study safety reports which was mild in nature and possibly related to the study drug and considered as completely resolved on 25 Aug 2014.

Subject number 10 was found to have (Increased SGPT levels) while evaluating the post study safety reports which was mild in nature and possibly related to the study drug and considered as completely resolved on 25 Aug 2014.

In this study, single dose of Ritonavir 100 mg capsule was well tolerated by this group of subjects under fasting conditions.

Withdrawal criteria

Subjects will be withdrawn from the study by the principal investigator or co-investigators for any

of the following reasons during the course of the study:

1. If the subject suffers from significant illness
2. If the subject requires concomitant medications which may interfere with pharmacokinetic of the study drug
3. If the subject has entered the study in violation of the inclusion and the exclusion criteria
4. If the subject is found to be non co-operative
5. If the subject decides to voluntarily dropout from the study

Note

- Any such subject withdrawals will be reported for reasons for withdrawal (if any).
- Medical examination of the subject will be done at the time of withdrawal / dropout.
- The plasma concentration data from subjects who are withdrawn due to adverse events will be presented, but will not be included in the statistical analysis.

There were no withdrawals in this study.

Check out process

After the completion of the study the subjects were checked- out. In the check out process the subjects undergo a medical check up to ensure that they are healthy even after participating in the study.

The study cycle was repeated after the washout period when the subjects are crossed over to other treatment.

Their post study medical check-up includes the blood test. Once the subjects finish giving their blood samples they are paid their compensation.

Washout period

Subsequent treatments should be separated by periods long enough to eliminate the previous dose before the next one (adequate wash out periods). There should at least 7 half lives of the drug as washout period between two treatments administrations.

In the present study drug administration in first period was followed by a washout period of 07 days before subjects were switched over to the other treatment in the second period.

Total duration of subject participation in the study

The total duration (excluding screening) of subject participation in a study was approximately 11 days including washout period of 07 days between each dosing. The duration of the total study with dates is explained in detail in below table.

| Activity | Date | No. of subjects |
|------------------|---------------------------|------------------------|
| Screening | 10 Jul 2014 – 30 Jul 2014 | 25 |
| Period-01 | | |
| Check-in | 31 Jul 2014 | 12 |
| Dosing | 01 Aug 2014 | 12 |
| Check-out | 02 Aug 2014 | 12 |
| Period-02 | | |
| Check-in | 08 Aug 2014 | 12 |
| Dosing | 09 Aug 2014 | 12 |
| Check-out | 10 Aug 2014 | 12 |

Accountability procedures for the investigational products:

Accountability was maintained for each unit of the investigational products by recording in appropriate forms. Drug store custodian and principal investigator were responsible for maintaining the accountability. The unused samples were sent back to the sponsor after completion of the study.

Subject compensation

The subjects were 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 was as follows:

| S. No. | Reasons of Withdrawal from the Study | Compensation |
|--------|--|---------------------------------------|
| 1. | Principal Investigator / Medical Officer withdraw the subjects from the study based on medical decision. | Full payment |
| 2. | After the initiation of the study, subject 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 illness/history. | No payment |

Bioanalytical phase

The estimation of Ritonavir in human plasma is carried out using LC/MS/MS method in Bioanalytical laboratory.

Analytical method details

Name of the Drug- Ritonavir

Name of the Analyte- Ritonavir

Instruments/equipments

The following instruments and equipments were used in the estimation of Ritonavir (analyte). Except LC/MS/MS, instruments and equipments of similar performance or equivalent configuration shall also be used for estimation of Ritonavir (analytes) using this analytical method.

Major equipment involved

Equipment Make Model

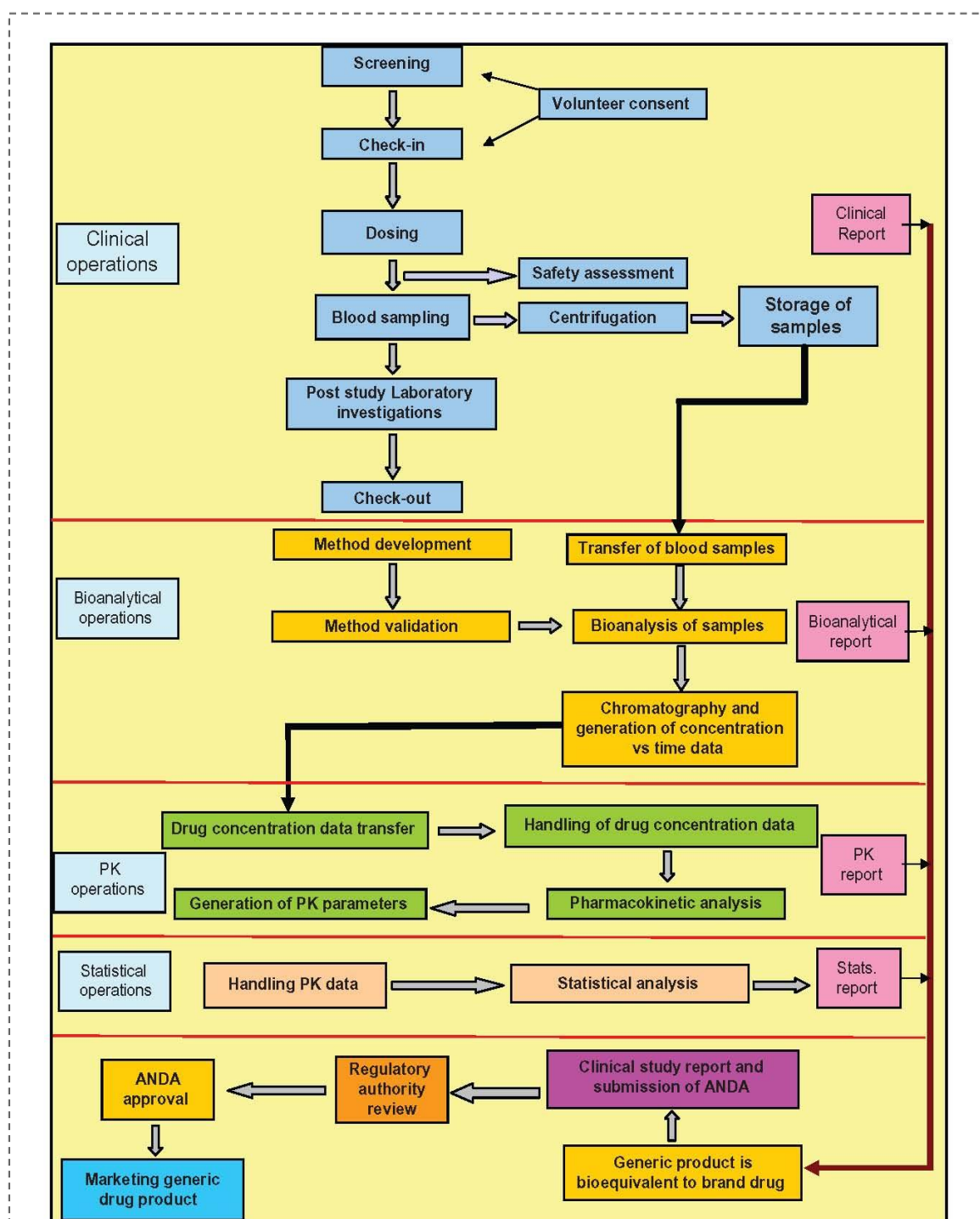
LC/MS/MS Applied Biosystems API 300

Pharmacokinetic and statistical phase

Based on the plasma concentrations of Ritonavir, the following pharmacokinetic parameters were calculated by using "Non-compartmental model" for Treatments A and B:

- Primary pharmacokinetic parameters: AUC_{0-t} , $AUC_{0-\infty}$, C_{max} .
- Secondary pharmacokinetic parameters: $AUC_{0-t}/AUC_{0-\infty}$, T_{max} , k_{el} and $t_{1/2}$.
- All pharmacokinetic analysis was carried out using WinNonlin Version 5.1.
- Statistical analysis was done from subject pharmacokinetic parameters using validated SAS[®] Version 9.1 software procedures.

Brief representation of work flow of bioavailability/bioequivalence study



Abbreviations: ANDA, abbreviated new drug application; PK, pharmacokinetics.

Results

Pharmacokinetic data

Mean (SD) of Pharmacokinetic Parameters of estimated for test product (A) and reference product (B) were as follows:

| Parameters (Units) | Un-transformed Data Mean \pm SD | |
|----------------------------------|--------------------------------------|-------------------------------|
| | Test Product (A) N=12 | Reference Product (B) N=12 |
| *T _{max} (hr) | 3.25 (1.00-4.50) | 4.50 (3.00-5.00) |
| C _{max} (ng/mL) | 1513.179 \pm 670.6014 | 1549.371 \pm 659.5110 |
| AUC _{0-t} (ng. hr/mL) | 12030.037 \pm 5460.2195 | 12233.229 \pm 7219.2410 |
| AUC _{0-inf} (ng. hr/mL) | 12759.075 \pm 5480.7590 | 12852.744 \pm 7416.2866 |
| K _{el} (1/hr) | 0.141 \pm 0.0366 | 0.134 \pm 0.0378 |
| t _{1/2} (hr) | 5.375 \pm 2.1283 | 5.602 \pm 1.7037 |
| AUC _{-%Extrap_obs} | 6.348 \pm 2.8361 | 5.206 \pm 1.3835 |

For T_{max}, Median (Min, Max) are presented.

Statistical data

The statistical results obtained for the drug during the fasting study were as follows:

Statistical Results of Assessment of Bioequivalence of Ritonavir under fasting Conditions (Ritonavir 100 mg capsules (Form A) with NORVIR® Ritonavir Capsules Soft Gelatin 100 mg (Form B))

| Parameters (Units) | Geometric Least Squares Means | | 90% Confidence Limits (%) | (A / B) % | Intra Subject CV % | Post- hoc Power (%) |
|-------------------------------------|----------------------------------|--------------------------|---------------------------------|--------------|--------------------------|------------------------------|
| | Test product (A) | Reference product (B) | (A vs. B) | | | |
| C _{max} (ng/mL) | 13993.530 | 1429.272 | 81.19-117.08 | 97.5 | 25.1 | 65.0 |
| AUC _{0-t} (ng. hr/mL) | 11005.510 | 10917.029 | 83.64-121.51 | 100.8 | 25.6 | 63.4 |
| AUC _{0-inf} (ng. hr/mL) | 11756.376 | 11517.708 | 85.39-122.01 | 102.1 | 24.5 | 67.0 |

The 90% CI for C_{max}, AUC_{0-t} and AUC_{0-∞} were not within 80.00-125.00% range.

Discussions

- In this study, single oral-dose of Ritonavir 100 mg Capsule was compared to NORVIR® 100 mg Soft Gelatin Capsule of Abbott Laboratories, USA, in normal, healthy, adult, male human subjects under fasting condition.
- In this study the pharmacokinetic parameters of test formulation was compared with the reference formulation in normal, healthy, adult, male human subjects under fasting condition.
- From the individual concentration vs time curves and the pharmacokinetic profiles, it was observed that the rate and extent of absorption parameters i.e. C_{max}, AUC_{0-t} and AUC_{0-∞} under fasting condition were found to be dissimilar for both reference and test treatment of Ritonavir.

The 90% CI of Ratio estimates of Ritonavir Capsules 100 mg versus NORVIR® Soft Gelatin Capsules 100 mg were [81.19-117.08], [83.64-121.51] and [85.39-122.01] for C_{max}, AUC_{0-t} and AUC_{0-∞}, respectively. All of these were within acceptable range of 80 to 125 % for C_{max}, AUC_{0-t} and AUC_{0-∞}.

Conclusion

As per the 12 subject's results, the test formulation - Ritonavir 100 mg Capsules was determined BIOEQUIVALENT to a single dose of reference formulation - NORVIR® 100 mg Soft Gelatin Capsules when both products were tested under fasting conditions in healthy, adult, human study participants.

Overall, a single dose of Ritonavir 100 mg Capsules and a single dose of NORVIR® 100 mg Soft Gelatin Capsules (Abbott), when given under fasting condition seem to have been equally tolerated by both groups comprising of 12 healthy, adult male human participants.

References

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