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Assessment of comparative bioavailability of Itraconazole capsule 100 mg under fasting conditions by average bioequivalence (ABE), population bioequivalence (PBE) and individual bioequivalence (IBE) approaches

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ABSTRACT

The assessment of interchangeability (prescribability and switchability) is one of the debatable topics in the generic drug industry. Currently, the question is whether we have an adequate assessment system for the evaluation of generic drug products. The objective of the study is to assess the comparative oral bioavailability of Itraconazole capsule 100mg after administering single dose to adult, healthy, human subjects in fasted state by different bioequivalence approaches like average bioequivalence (ABE), population bioequivalence (PBE) and individual bioequivalence (IBE) and to monitor the safety of study subjects. An open-label, balanced, randomized, two-treatment, two-sequence, four-period, crossover, single-dose comparative oral bioavailability study was conducted in sixteen healthy, adult, human subjects in a fasted state. Test formulation, Itraconazole capsule 100mg, and reference formulation, SPORANOX® (Itraconazole) capsule 100mg, were administered in a fasted state. The test formulation, Itraconazole capsule 100mg, showed bio-inequivalent against reference SPORANOX® (Itraconazole) capsule 100mg in study subjects under a fasted state. Also, the test formulation exhibited a similar safety and tolerability profile compared to the reference formulation. There was no report of serious adverse events (SAEs) and deaths in the study. The test formulation was found to be bio-inequivalent to reference formulation in the study subjects under a fasted state by estimating different bioequivalence approaches like average bioequivalence, population bioequivalence, and individual bioequivalence.

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INTRODUCTION

The generic drug products are very vital for the treatment of patients. They have the advantage of the same therapeutic benefit as the innovator product with a substantial reduction in the cost. Now the question arises about how the generic drug product can be interchanged (prescribability and switchability) with the innovator product/ standard drug and how this gets assessed. In general, the generic product and the innovator product are assessed in a comparative bioavailability study by the average

bioequivalence (ABE) evaluation method. In the ABE approach, the 90% confidence interval of the relative mean (Geometric mean) of the test formulations with that of reference formulation for Ln-transformed transformed pharmacokinetic parameters (C_{max} and AUCs) should be within 80.00% to 125.00% for a generic product to establish bioequivalence. When the drug which are highly variable in nature and narrow therapeutic index drug are dealt by ABE approach, the typical 80.00% to 125.00% confidence limits are allowed to expand or narrowed down based on the characteristic of the drug products. These modifications in bioequivalence assessment raise the concern of whether ABE assessment is the right tool for the evaluation and whether this method adequately addresses interchangeability. ABE method considers only population mean for the assessment, whereas in the case of population bioequivalence (PBE) assessment, it considers inter-subject variability along with population mean, and individual bioequivalence (IBE) approach consider within-subject, and subject-by-formulation variances along with population mean for the assessment. Hence PBE approach addresses the prescribability, and the IBE approach addresses switchability (Micheal and Balamurali, 2015).

In order to understand the study outcome by applying the different bioequivalence assessment approaches in a clinical pharmacokinetic study, a model study with Itraconazole capsule 100mg was chosen. Thus, the objective of this clinical pharmacokinetic study was to evaluate the comparative oral bioavailability of newly established Itraconazole capsule 100mg (Strides Arcolab Limited, India) with that of the standard drug SPORANOX® (Itraconazole) capsule 100mg upon administering single dose to adult, healthy, human subjects under fasted state by different bioequivalence approaches like average bioequivalence, population bioequivalence, and individual bioequivalence and to monitor the safety of subjects.

As test itraconazole 100mg capsule, Strides Arcolab Limited, India is a generic version of SPORANOX®, USA, test formulation is qualitatively and quantitatively, similar to the standard drug.

This study was executed to understand the test formulation, Itraconazole capsule 100mg in-vivo behavior against the standard formulation, SPORANOX® (Itraconazole) capsule 100mg. This was a pilot study and not planned for any regulatory submission purpose. This study was planned as per the draft product-specific guidance from (USFDA, 2011). As it was a pilot study, only parent analyte was quantified for the pharmacokinetic and statistical evaluation.

The brief description of Itraconazole is mentioned below,

1. Azole antifungal agent
2. 1:1:1:1 racemic mixture of four diastereomers Molecular formula: C
3. Molecular weight: 705.64
4. pKa: 3.70
5. Partition coefficient: 5.66 at pH 8.1
6. White to slightly yellowish powder
7. Insoluble in water, very slightly soluble in alcohol, and freely soluble in dichloromethane (Sporanox® PIL, 2018; Sporanox SPC, 2013; Pr Sporanox®, 2019).

The chemical structure of Itraconazole is given in Figure 1.

Pharmacokinetic profiles of Itraconazole

C_{max} – within 2 to 5 hours

Absolute oral bioavailability - 55%

Steady State attainment - within about 15 days

Terminal half-life - 16 to 28 hours after a single dose and increases to 34 to 42 hours with repeated dosing (Sporanox® PIL, 2018; Sporanox SPC, 2013; Pr Sporanox®, 2019).

MATERIALS AND METHODS

Ethical considerations

The study was started only after receiving an approval from the Institutional ethics committee (IEC), and from each subject, written informed consent was obtained. The study procedure was explained to the subjects in their respective native languages. The study was conducted as per the Good Clinical Practices, Declaration of Helsinki, and applicable requirements of principles of Good Laboratory Practices (WHO, 2009; OECD, 1977; ICMR, 2017).

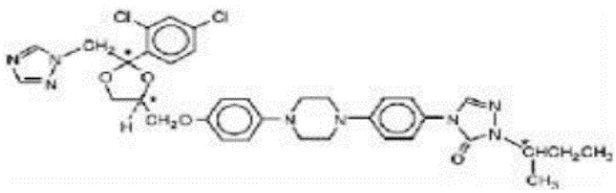

Figure 1: Chemical structure of Itraconazole

Study design

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Table 1: Demographic data of study subjects

Demographic characteristics	Mean	Min.	Max.
Height (cm)	168.9	159.3	181.8
Weight (kg)	57.9	51.3	69.2
BMI (kg / m^2)	20.30	18.53	24.04
Age (years)	29	20	39

BMI = body mass index, Min. = Minimum, Max. = Maximum

Table 2: Pharmacokinetic parameters of Itraconazole in 13 study subjects in fasted state

PK parameters (Arithmetic mean ± SD)	Test Formulation (T1)	Test Formulation (T2)
C_{max} (ng/mL)	90.36 ± 61.58	80.77 ± 47.38
AUC_{0-t} (ng h/mL)	1377.41 ± 963.64	1175.79 ± 730.85
$AUC_{0-\infty}$ (ng h/mL)	1441.57 ± 1003.64	1241.84 ± 788.19
T_{max} (h)	3.5 (2.0 – 5.5)	3.65 (2.0 – 5.0)
K_{el}	0.03 ± 0.01	0.03 ± 0.01
$t_{\frac{1}{2}}$ (h)	26.78 ± 13.84	24.60 ± 9.12
PK parameters (Arithmetic mean ± SD)	**Reference Formulation (R1)**	**Reference Formulation (R2)**
C_{max} (ng/mL)	104.76 ± 73.58	114.59 ± 67.30
AUC_{0-t} (ng h/mL)	1490.14 ± 1139.08	1642.13 ± 1034.13
$AUC_{0-\infty}$ (ng h/mL)	1546.95 ± 1189.12	1726.16 ± 1114.86
T_{max} (h)	3.5 (2.0 – 5.0)	3.46 (2.0 – 5.5)
K_{el}	0.03 ± 0.01	0.03 ± 0.01
$t_{\frac{1}{2}}$ (h)	23.47 ± 11.46	26.83 ± 12.07

Table 3: Bioequivalence summary - ABE, PBE, and IBE approaches

ABE evaluation variances	Test/reference values for log-transformed	90% Confidence interval
Cmax (ng/mL)	76.49	63.01 - 92.84
AUC0–t (ng h/mL)	82.14	67.67 - 99.72
AUC0–∞ (ng h/mL)	82.72	68.19 - 100.34
	Pass or fail ABE	Fail - Cmax and AUC
PBE evaluation variances	**Linearized point estimate**	**95% upper confidence bound**
Cmax (ng/mL)	76.49	0.0003 (Reference scale)
AUC0–t (ng h/mL)	82.14	-0.212 (Reference scale)
AUC0–∞ (ng h/mL)	82.72	-0.2187 (Reference scale)
	Pass or fail PBE	Cmax - Fail & AUC passes
IBE evaluation variances	**Linearized point estimate**	**95% upper confidence bound**
Cmax (ng/mL)	76.49	0.1559 (Reference scale)
AUC0–t (ng h/mL)	82.14	-0.0833 (Reference scale)
AUC0–∞ (ng h/mL)	82.72	-0.0792 (Reference scale)
	Pass or fail IBE	Cmax - Fail & AUC passes

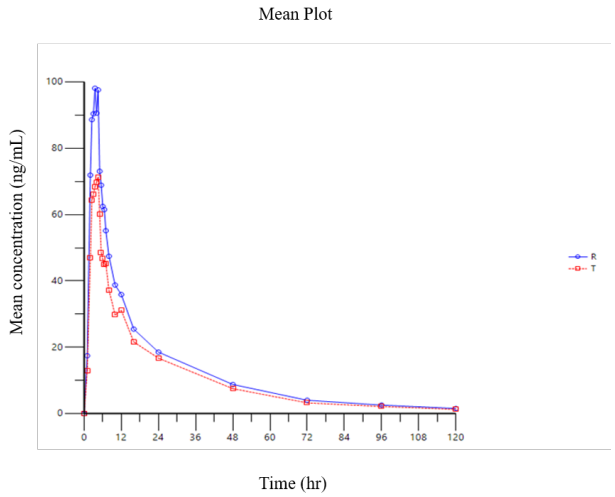

Figure 2: Mean plasma drug concentration–time curve

This was a balanced, randomized, open-label, two-sequence, single-dose, two-treatment, four-period, fully replicate crossover comparative oral bioavailability study. As it's a complete replicated design study, all the study subjects were administered the test formulation in two periods and the reference formulation in the other two periods as per the assigned randomization schedule. The study subjects were randomly assigned to receive tests or reference products in a fasted state.

Study procedure

Housing - At least 11 hours before dosing to at least 24 hours post-dose

Ambulatory sample collection - 48.0, 72.0, 96.0 and 120.0 hours post-dose

Fasting - At least 10 hours before dosing to at least 4 hours post-dose

Water intake - restricted at least from 1 hour before dosing until 2 hours post-dosing (except for water used for drug administration).

Posture restriction - remained seated for 4 hours post-dose & thereafter, to ambulate freely

Any prescription medications - prohibited within 14days prior to dosing and throughout the study

Over the counter (OTC) products, herbal medications - prohibited within 7 days prior to dosing and throughout the study

The study drug was administered with 240mL of water in each period. The dosing activities were monitored by the trained study team, and the dosing compliance was ensured.

In each period, 22 venous blood samples were collected at 0.0, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 8.0, 10.0, 12.0, 16.0, 24.0, 48.0, 72.0, 96.0 and 120.0 hours post-dose in labeled K_3 EDTA vacutainers for the evaluation of pharmacokinetic parameters. The pre-dose sample was collected within 1 hour before drug administration. The samples were centrifuged to separate the plasma at 3000rpm for a period of fifteen minutes at 4°C. After separation of plasma, they were transferred in labelled tubes, which were collected in duplicate and stored at -20º± 10ºC in a deep freezer at the clinical site. Till the study subjects' samples were shifted to bioanalytical laboratories; they were kept in the clinical site.

Study population

1. Healthy subjects of age between 18 and 45 years (both inclusive)
2. BMI between 18.5 and 24.9 in kg/ m2

The subjects were chosen on the outcome of laboratory evaluations during screening, clinical examination, medical history, chest X-ray, and ECG recordings. For the reason of numerous socio-cultural circumstances, a female subject could not be convinced to stay at the clinical facility. Also, generally, females in a country like India are likely to have lesser hemoglobin levels. Further, the drug under investigation is not recognized to have a gender-specific pharmacokinetic profile. Therefore, the study was conducted by enrolling male subjects. The study subjects were chosen based on their inclusion and exclusion eligibility criteria. This study was conducted according to requirements of applicable regulatory agencies (E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1), 2018; Helsinki, 2008; bioanalytical method validation, 2018).

The study protocol and informed consent documents were submitted for an independent ethics committee (IEC) review and approval. Subjects were enrolled into the study only upon receipt of IEC approval.

Safety

Safety and tolerability of test and reference formulation for the enrolled subjects were evaluated in the study by the following ways.

1. Monitoring adverse events (AEs)
2. Standard clinical laboratory tests (clinical biochemistry, urinalysis, and hematology)
3. Physical examinations
4. Vital signs
5. 12-lead electrocardiograms (ECGs)

6. Post-study safety follow-up

Bioanalytic methods

The bioanalytical method (HPLC-MS/MS bioanalytical method) was validated as per the recommendation from the FDA (bioanalytical method validation, 2018). The assay was carried out at Jeevan Scientific Technology Limited, Hyderabad, which was specific for the determination of Itraconazole.

Pharmacokinetic analysis

The following pharmacokinetic parameters were estimated for Itraconazole using a non-compartmental model analysis by using WinNonlin® professional software (Version 5.3, Pharsight Corporation, USA)

1. C_{max} - Maximum measured plasma concentration over the time span specified.
2. AUC_{0-t} - Area under the plasma concentration curve from administration to last observed concentration at time t
3. $AUC_{0-\infty}$ - Area under the plasma concentration curve extrapolated to infinite time
4. $AUC_{0-t}/AUC_{0-\infty}$ - % AUC extrapolated area
5. T_{max} - Time until C
6. K_{el} - Terminal rate constant
7. $t_{\frac{1}{2}}$ - Plasma concentration half-life

Statistical analysis

Statistical analyses were performed on the pharmacokinetic parameters using the General Linear Models Procedure (PROC GLM) of SAS Software (SAS Institute, Cary, NC). The model tests for treatment effects in the parameter means at an alpha level of 0.05. The parameters: T_{max}, K_{el}, and THalf were analyzed statistically using the non-transformed data. The natural log-transformed parameters: $LNAUC_{0-t}$, $LNAUC_{0-\infty}$, and LNC_{max} were also analyzed. Tests were performed to analyze for statistically significant differences in the pharmacokinetic parameters and to determine the test to reference ratios of the pharmacokinetic parameters using Least Squares Means. Ninety (90%) percent confidence intervals were constructed using the two one-sided tests procedure to assess average bioequivalence between the two formulations. The primary pharmacokinetic variables for the assessment of bioequivalence were C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$. Statistical analysis of primary pharmacokinetic parameters C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$ of Itraconazole was carried out using average bioequivalence, population bioequivalence, and individual bioequivalence approaches.

RESULTS AND DISCUSSION

Study subjects' demographic data

A total of 13 study subjects were enrolled into the study, and their demographic characteristics were comparable and presented in Table 1.

Pharmacokinetic assessment

The mean plasma concentrations of a single dose of test formulation, Itraconazole capsule 100mg, and the standard formulation, (Sporanox® PIL, 2018) (Itraconazole) capsule 100mg concentrations in a fasted state is presented in Figure 2.

The pharmacokinetic parameters of a single dose of test formulation, Itraconazole capsule 100mg, and the standard formulation, (Sporanox® PIL, 2018) (Itraconazole) capsule 100mg concentrations in a fasted state is presented in Table 2.

Bioequivalence Assessment

The bioequivalence summary of a single dose of test formulation, Itraconazole capsule 100mg, and the standard formulation, (Sporanox® PIL, 2018) (Itraconazole) capsule 100mg concentrations in a fasted state is presented in Table 3.

Safety and tolerability profile

In total, 13 subjects received the investigational drugs in all the periods. Both the formulations (test and reference) were well tolerated. There were no deaths reported during the study. The safety profile of test Itraconazole capsule 100mg was found comparable to the standard drug (Sporanox® PIL, 2018) (Itraconazole) capsule 100mg in a fasted state.

Though there has been a significant improvement in pharmaceutical research, the change is inevitable. Generic drugs play a significant role in treating patients all across the globe. The significant advantages of generic drugs are lower cost and providing the same efficacy as innovator products. ABE approach is the gold standard bioequivalence evaluation method. Whether the drug belongs to a highly variable drug category or a narrow therapeutic index drug category, the same ABE approach is used for the evaluation, and the confidence limit is adjusted to make sure that the particular category of drug is evaluated as applicable. If the drug of interest for the evaluation is a highly variable drug category, 90% confidence internal will be applied, and the confidence limit will be widened. If the drug of interest for the evaluation is a narrow therapeutic index drug category, 90% confidence internal will be applied, and the confidence limit will be tightened. Though there have been numerous

changes in the usage of the ABE approach, there were many discussions and doubts about using this approach for the narrow therapeutic index drug category and highly variable drug category. Then the alternative approaches like PBE and IBE approaches emerge (Zariffa and Patterson, 2001; Endrenyi and Midha, 1998; Tothfalusi and Endrenyi, 2003; Wijnand, 2003; Dragojevic-Simic *et al.*, 2017).

From the available literatures, it is very well established that itraconazole has high intra-subject variability. Due to its high intra-subject variability, the available studies were conducted by partial/ complete replicate design (SBOA, 2010; Suarez-Kurtz *et al.*, 1999; Dragojević-Simić *et al.*, 2018).

This clinical pharmacokinetic study was undertaken to assess the comparative oral bioavailability of test formulation, Itraconazole capsule 100mg against the standard formulation, (Sporanox® PIL, 2018) (Itraconazole) capsule 100mg in a fasted state. In this study, the study outcomes were assessed by ABE approach along with PBE and IBE approaches. From the mean plasma concentration of test Itraconazole capsule 100mg, it was found to be not comparable to the standard drug (Sporanox® PIL, 2018) (Itraconazole) capsule 100mg. Form the mean plasma drug concentration. It is quite evident that the test formulation release was slower than the reference formulation, and the same was observed with the pharmacokinetic parameters also. From the bioequivalence summary data, it is very clear that the test formulation is bio-inequivalent to the reference formulation. All the bioequivalence approaches assessment shown the same outcome. There was no death, and serious adverse events (SAEs) reported in the study. Both the test and reference formulation exhibited comparable safety and tolerability profiles.

The objective of this clinical pharmacokinetic study was to understand the test formulation behavior and not to prove bioequivalence. Moreover, this was a pilot study. Based on the study outcome, it was quite apparent that the used test product must undergo re-development to meet bioequivalence with the standard drug.

CONCLUSION

It is apparent from the study outcome that test formulation is bio-inequivalent to the reference formulation by the ABE approach, and the same results were observed by evaluating the PBE approach and IBE approach. The primary pharmacokinetic parameters C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$ were failing in ABE method, and AUC_{0-t}, $AUC_{0-\infty}$ were passing though C_{max} was failing in PBE and IBE method. Certainly, the study outcome clearly communicates that the study outcome differs in different bioequivalence approach evaluation as the considerations for the approaches are different.

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