Comment Response Document

S.No.	Reviewer's Comment	Author's Response
	Reviewer #1: **Scientific Quality:** Grade B (Very good) **Language Quality:** Grade B (Minor language polishing) **Conclusion:** Accept (General priority) **Specific Comments to Authors:** The paper reports a bioequivalence study of a new formulation of itraconazole compared to an existing formulation. The study was performed using a three-way cross-over design in healthy volunteers and PK data was determined using non-compartmental calculations. Bioequivalence was assessed by ANOVA on the log-transformed AUC data. Thus, the study was carried out using standard methodology for this type of study and the derived parameters reported in a conventional manner. There are several issues with the report that require amendment / revision before the paper can be published.	
	1.The study appears to be a BE study for the purpose of drug registration. If this is the case, it should be noted in the Conflict-of-Interest statement at the end of the paper.	Dear Reviewer: We have mentioned in source of support: The test product Fixtral SB was sponsored by Dr. Reddy's Laboratories Limited.
	2. Somewhat surprisingly there is no mention of the methodology used for the determination of plasma drug and metabolite concentrations. This should report sample preparation, instrument(s) used, chemical methodology and the accuracy, precision, and detection limits of the assay method.	Dear Reviewer: We have added the complete procedure in the manuscript under blood sampling. The added text is highlighted in yellow.
	3. It is not clear why R1 was used in the study when the new formulation appears to have been established as bioequivalent to it. Would it have been better to report the data for R1 as dose corrected so that the reported values are comparable between test, R1 and R2?	Dear Reviewer: R1 used in the study as Conventional formulation and test product has different dose strengths and thus cannot be BE. Both test and conventional formulation R1 are same strengths so dose correction not possible

	4. Even though the authors report that the ratio of AUC/MIC was calculated for test (T) and reference (R1) at different levels of MIC up to maximum 16 mcg/mL, this data does not seem to appear anywhere in the paper. Furthermore, statistical comparisons between formulations are not reported other than the bare statement that the two formulations were equivalent.	Dear Reviewer: It is given in section statistical analysis. The text is highlighted in yellow
	5. With respect to the demographic data the number of males and females is not stated separately nor are their ages, weight etc reported. Perhaps a separate table reporting these demographic data is in order. If the weight and height are reported, is it necessary to report BMI, since it is not an independent variable or alternatively if BMI is reported then weight and height would seem superfluous.	Dear Reviewer: we have updated the details on gender ratio in results under demographic characteristics. The text is highlighted in yellow We have also given all the details of BMI and height and weight also, but as suggested we have now removed weight and height details and retained only age and BMI mean and SD values, the text is highlighted
	6. The units for the value of Cmax are not stated in the text or in tables 2 and 3.	
	In the absence of the assay methodology the reported precision of measurements is overstated. Indeed, it is doubtful if any analytical method has the precision implied by the reporting of the data, particularly at ng/ml levels.	Dear Reviewer:: We have added the units for Cmax in both the tables and texts (at all applicable places). The changes are highlighted. We have a validated assay methodology with linearity range was 0.504ng/mL to 402.041 ng/mL for Itraconzaole
	7. Why was the data from one subject not included in the analysis?	Dear Reviewer: We have clearly mentioned that from 54 subjects considered for clinical analysis, 3 subjects were withdrawn from the study due to AEs. Though the data of subjects, completing at least 2 periods of the study (provided subject has received the test product in any one of the 2 periods attended) were considered for PK and statistical

		analysis. Hence, data of 52 subjects were considered for the statistical analysis of test vs reference product (R2). **Also please note that: Subject no. 36 was withdrawn from the study in period I before dosing (Vomiting reported as AE) and replaced with additional enrolled subject no. 55 i.e. (E1) as subject no. 36.**
	Reviewer #2: **Scientific Quality:** Grade C (Good) **Language Quality:** Grade B (Minor language polishing) **Conclusion:** Major revision **Specific Comments to Authors:** This study reports on what seems to be a formal BE study done for regulatory purposes. T product is a generic formulation (T) developed after an approved formulation of intraconazole that was designed in order to achieve improved absolute oral bioavailability (R2) - i.e., both T and R2 are "suprabioavailable" formulations of itraconazole, and T (generic) is compared to R2 (reference) in a formal single-dose, first-order cross-over study under fed conditions. The study includes also a further "Reference", which is a "conventional" oral formulation of itraconazole. The dose of T is 100 mg and is compared to 100 mg of R2 (i.e., two suprabioavailable formulations are compared on equivalent doses). The dose of R1 is 200 mg - i.e., suprabioavailable formulations (which has been previously shown for R2 formulation) are approximately twice more bioavailable, hence the approved dose is half the dose of the conventional formulation. With 3 treatments (T, R1, R2) - the study is hence 3 treatment 3 period cross over study. Generally, the addressed topic is of interest, but there are several flaws in this manuscript and it requires a major revision. 1. Some English polishing is needed.	Dear Reviewer: We have tried our best to refine the language

	2. Abstract is not straightforward to understand. It is a bit confusing. It mentions "3 treatments", but then mentions and reports only T vs. R2 results etc. _ it should be re-written in a way which will make it straightforwardly clear: There are 3 treatments, T is suprabioavailable and is tested for BE vs. a suprabioavailable reference (one referene treatment), There is another reference treatment - a conventional formualtion. T is NOT compared to thie reference for PK BE, but for a pharmacodynamic parameter AUC/MIC.	Dear Reviewer: We have modified the abstract –
	3. Figure 1 depicts subjects flaw and study design - but it is again confusing (a bit): a) if this was a 3 treatment 3 period single dose first-order cross-over, than it should have had 6 sequences - and 54 subjects were randomized 9 to each sequence (Williams design for 3 treatments). This should be explicitly stated. 3. Concentration-time curves for the 3 treatments should be clearly graphically displayed. 4. A tabular or graphical representation of proportions achieving the "critical" AUC/MIC ratio per time points should be shown for T and R1.	Dear Reviewer: A 3way BE study was performed with Fixtral SB 100mg (Supra bioavailable test product) vs. Lozanoc, Supra bioavailable Reference product (2 capsules of 50mg) and 200mg conventional Itraconazole formulation. Dear Reviewer: We have not mentioned about the BE results of 200mg conventional Itraconazole formulation and AUC/MIC ratio for conventional Itraconazole.
	5. In Results - this is a standard cross-over study. the so-called "period effect" or "sequence effect" or "significant treatment effect" - are irrelevant info. What matters is a) data summary; b) formal BE tests/ratios. I assume that - in line with the standards of cross over PK studies, subjects with pre-dose levels >5% of the previous period peak -were excluded (yes? should be stated explicitly). If so (and this is how it should be) - there is no carry-over - and one is not concerned with anything else.	Dear Reviewer: In our study, no pre-dose levels >5% occurred for any of the subjects, which concludes no carry over effects

	6. Methods - clearly describe design (as mentioned), declare the rationale for the number of subjects; describe the bioanalytical method. While the method for the analysis of PK BE is rather clear (provide the proc mixed code), the method to analyze proportions is not really clear. The fact is - proportions from a cross-over design can also be analyzed just as the continuous outcome - a mixed model with subjects nested in sequence, with a binary distribution and a logit link (with treatment, period, sequence and subjects nested in sequence as fixed effects, or with subjects as a random effect).	Dear Reviewer: We have given how the sample size was determined in results, it is highlighted in yellow. Analysis of subject samples for estimation of Itraconazole was measured by a validated LC-MS/MS analytical method. SAS®PROC MIXED procedure was used for ANOVA and the estimation of least squares means (LSMs) differences (Test (T)-Reference (R2)) formulations on the ln-transformed PK parameters Cmax, AUC0-t and AUC0-inf at an α level of 0.05. The corresponding standard errors of the differences were also computed. The analysis of variance model included sequence, period and treatment as fixed factors and subject nested within sequence as random factor. An ANOVA model was used to analyze each of the parameters. The significance of the sequence effect was tested using the subject nested within sequences as the error term. All other main effects were tested against the residual error (mean square error) from the ANOVA model as the error term. Each analysis of variance also included calculation of least-square means, adjusted differences between formulation means and the standard error associated with these differences.
	7.IN BE studies, Cmax is a standard "primary outcome"..why not here? (i.e, T and R are BE if their peak (cmax) and total (AUCt) exposures are equivalent).	Dear Reviewer: Please see the response below

	8. AUC0-t is not AUC from 0 to the last measurable concentration, but to the last sampling time. 9. First table in the mansucrtipt should be one showing summary PK and PD data for T, R1, R2. 10. the next one should show formal BE tests, or comparisons in respect to the proportions. 11. Why compare AUC/MIC vs. "a conventional" formualation and not R2? 12. The current Table 1 and Table 2 seem to be discordant...the numbers do not seem to match - check it! 13. In Table 1, CIs for the Cmax GMR are erroneous - correct.	Dear Reviewer: It is corrected and highlighted Dear Reviewer: Conventional formulation and test product has different dose strengths and thus can not be BE. Thus to demonstrate comparative PD effect between conventional formulation and test product, AUC/MIC data analysis was done to demonstrate that both arms have ratio > 25-50. This means both products will have similar efficacy. However R2 and test product are BE, thus additional PD comparison not required Dear Reviewer: It is all corrected and highlighted Dear Reviewer: It is corrected and highlighted