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Research Article

Soluble Itraconazole in Tablet Form Using Disordered Drug Delivery Approach: Critical Scale-up Considerations and Bio-equivalence Studies

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Abstract. The present research work explores formulation design, critical scale-up considerations and bio-equivalence studies of soluble itraconazole (ITZ) in a tablet form using disordered drug delivery approach. Disordered system of ITZ with a lower viscosity grade of hydroxypropyl methyl cellulose (Pharmacoat 603) was developed for the first time and extensively characterised at three different stages, namely development of glass system, pellet coating and tablet compression using advanced analytical techniques. Complete molecular embedment of ITZ resulting in amorphisation was observed and found to be sustained until end of the real-time and accelerated stability studies. Developed formulation exhibited comparative *in vitro* dissolution profile (similarity factor >70) with reference product (Sporanox, Janssen Pharmaceutica) in simulated gastric fluid without enzymes. Formulation was scaled up in three batches (50,000 tablets/batch) with detailed validation of critical process parameters using process capability index method. Critical scale-up considerations like control of residual solvent content, effect of pellet size on dissolution, process variables in pellet coating, compressibility of coated pellets and cushioning effect required for desired compressibility were thoroughly discussed. Bioequivalence study of single dose of test and reference product in seven healthy human volunteers under fed condition exhibited significant bioequivalence with results (AUC_{last} and AUC_{∞}) lying between 90% confidence interval. With increase in number of subjects to 24, a significant effect on pharmacokinetic parameters of both reference as well as developed ITZ tablets was observed.

KEY WORDS: bio-equivalence; disordered drug delivery; itraconazole; scale-up; validation.

INTRODUCTION

In comparison to Biopharmaceutical Classification System (BCS) class I drugs (high solubility), BCS class II and IV drugs (low solubility) are known to be more recalcitrant in nature, owing to their poor solubility and atypical *in vivo* consequences like incomplete bioavailability, intra-/inter-patient variability in pharmacokinetic parameters, incomplete drug release and significant food effects (1,2). In addition to this, poorly soluble drugs suffer from numerous formulation difficulties, like limited drug delivery strategies, reproducibility in drug release, altered dissolution profiles in scale-up and scale-down of formulation and poor predictability of *in vitro*/*in vivo* correlations (3–6).

Disordered drug delivery approach utilizes an innovative concept of molecular trapping in a high-energy non-crystalline state (7). The consequential amorphous or disordered composite of drug offers significant boost in solubility and bioavailability by many folds in comparison with its crystalline forms (7,8). They have a molecular structure like liquids and a macroscopic structure like solids thereby providing the patient with a convenient dosage form. Understanding the extent of molecular movement, molecular relaxation and molecular distribution in disordered systems with advanced analytical techniques allowed further growth with respect to reproducible and industrially scalable manufacturing of these systems (7). Molecular matrix of poorly soluble drug like itraconazole (ITZ) with hydrophilic polymer provides a very interesting system design for fabrication of disordered drug delivery system.

ITZ is a BCS class II, potent antifungal agent of the triazole class. It has an extremely low aqueous solubility (<1 μg/ml) and poor dissolution rate in the GI tract (9–11). As a consequence of its poor solubility profile, it shows large inter-individual differences in bioavailability after oral administration (11,12). Currently, ITZ is marketed as Sporanox® capsules by Janssen Pharmaceutica. Sporanox® capsules contain ITZ coated on sugar spheres. Prior studies pertaining to understanding the use of ITZ capsules in neutropenic patients for antifungal prophylaxis reported that >40% of patients exhibited very low plasma ITZ levels after 2 weeks of daily dosing (13,14). ITZ oral solution and IV injection were developed as newer

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formulations to surmount the pharmacokinetic limitations of marketed capsule formulation (15). These newer formulations exhibited enhanced absorption and steady plasma level. As ITZ shows a dose-dependent pharmacokinetics, variability in pharmacokinetic parameters in fed and fasted states, intra- as well as inter-individual variability and multiple drug interactions, it is always challenging to determine a perfect dosing regimen in patient suffering from critical fungal infections (16,17).

The thought of developing a simple and scalable tablet formulation was originated considering pharmacokinetic deficiencies and high cost involved in capsule formulation. Some excellent research work on ITZ showing potential of concentration enhancing polymeric solid dispersions and application of hot melt extrusion technique-based solid dispersions and interested findings reported thereof in prior art further boosted us to work on fundamental and mechanistic behaviour of ITZ amorphous systems (18–24). However to the best of our knowledge, use of lower viscosity polymer for the said purpose, critical scale-up considerations, detailed validation of scale-up studies and human bioequivalence studies of ITZ disordered systems in tablet form is not explored yet.

The present research work deals with enhancement in solubility of ITZ using disordered drug delivery technology by fabricating a glass system of ITZ in lower viscosity grade hydroxypropyl methyl cellulose (HPMC-3 cps) aiding ease in processability. The glass system is further characterized to understand the mechanistic reasons for solubilization. An important objective is, however, to scale up the lab design of disordered drug delivery with simultaneous process validation to achieve a bioequivalent product in tablet form with high industrial significance.

MATERIALS

ITZ was procured from Ultratech Ltd., India. HPMC-3 cps (Pharmacoat®) and Sugar pellets (25/30 mesh, 30/35 mesh and 35/40 mesh ASTM) were purchased from Signet Chemical Corporation, India. All other tableting excipients were procured from Signet Chemical Corporation, India. Solvents used for lab purposes were of analytical reagent grade/high-performance liquid chromatography (HPLC) grade depending on the application, and those used for scale-up purposes were of commercial grade. Ammonium acetate GR grade was procured from Merck, India.

METHODS

Quantitative Analysis of ITZ

A stability indicating RP-HPLC (Jasco Intelligent system with Jasco PU 980 Intelligent HPLC pump, Jasco UV-975 Intelligent UV/VIS detector) method was developed and validated for quantitative determination of ITZ. The mobile phase composition was ammonium acetate (0.5%, *w/v*): methanol/acetonitrile (25:25:50). The column used was RP-18 LiChrospher (25 cm×4.6 mm, 5 μm) under isocratic conditions at flow rate of 1.4 ml/min. The volume of injection was 20 μl, and the determination of ITZ was carried out at 258 nm. A separate gradient RP-HPLC method for analysis of related substances was developed and validated. The mobile phase composition was *A*=27.2 g/l solution of tetrabutylammonium hydrogen sulphate R and B = acetonitrile. The column used was Hypersil BDS (100×4.0 mm, 3 μ) under gradient conditions (Supplementary Table 1) at flow rate of 1.5 ml/min. The volume of injection was 10 μl, and the analysis was carried out at 225 nm.

Formulation Development

Formulation development of ITZ disordered drug delivery system was initiated with experimental batches at lab scale. Various formulation variables involved in each stage were optimized for better product attributes. Figure 1 summarises sequential processing stages and respective formulation variables involved in product development. Lab-scale development is explained concisely as authors would like to give more stress on in depth characterisation, industrial scale-up and bioequivalence studies of developed formulation.

Disordered Drug Delivery System at Lab Scale

Formulation of Glass System

Lab-scale formulation trials of disordered delivery system of ITZ were initiated with molecular entrapment of ITZ in low viscosity HPMC-3 cps (Pharmacoat 603®) using film casting technique. ITZ was dissolved in methylene chloride to form solution A (1 part of ITZ: five parts of methylene chloride). HPMC-3 cps (Pharmacoat 603®) was dispersed in isopropyl alcohol and dissolved in methylene chloride (one part HPMC: four parts isopropyl alcohol and four parts methylene chloride) to form solution B. The proportion of ITZ/HPMC was varied from 1.0:0.5 to 1.0:5.0 parts by weight. The casted films were dried at 60°C in hot air oven for 2 h.

Coating of Glass System on Pellets

ITZ/HPMC glass system was further loaded on sugar pellets on a Wurster air suspension coater (Anish Pharma, India). Effect of ratio of ITZ/HPMC on *in vitro* release of ITZ after pellet coating was evaluated as multi-unit particulate systems (MUPS) offer different surface area and dissolution dynamics than glass system in the form of film. Ratio of ITZ/HPMC was varied from 1.0:0.5 to 1.0:2.5, and coated pellets were evaluated for *in vitro* drug release in simulated gastric fluid, pH 1.2. Core sugar pellets of various dimensions viz. 25/30 mesh, 30/35 mesh and 35/40 mesh ASTM were tried as a support material. Pellets were uniformly coated with prepared solution in order to deposit a thin layer of glass system. Amount of core pellets were optimized to achieve similar dissolution profile as of innovator product, keeping ITZ/HPMC ratio and pellet core dimensions constant.

Formulation of Tablets

Many strategies were tried to compress tablets of MUPS. To mention every trial is not in the scope of this paper. Briefly the MUPS were crushed in a domestic mixer using a high shear blade at maximum speed. The crushed pellets (equivalent to 100 mg itraconazole) were then mixed with dummy granules fabricated from lactose, microcrystalline cellulose, cross-carmellose sodium and polyvinyl pyrrolidone. Other excipients like low substituted hydroxypropyl cellulose LH-11, sodium starch glycholate (type A), magnesium stearate

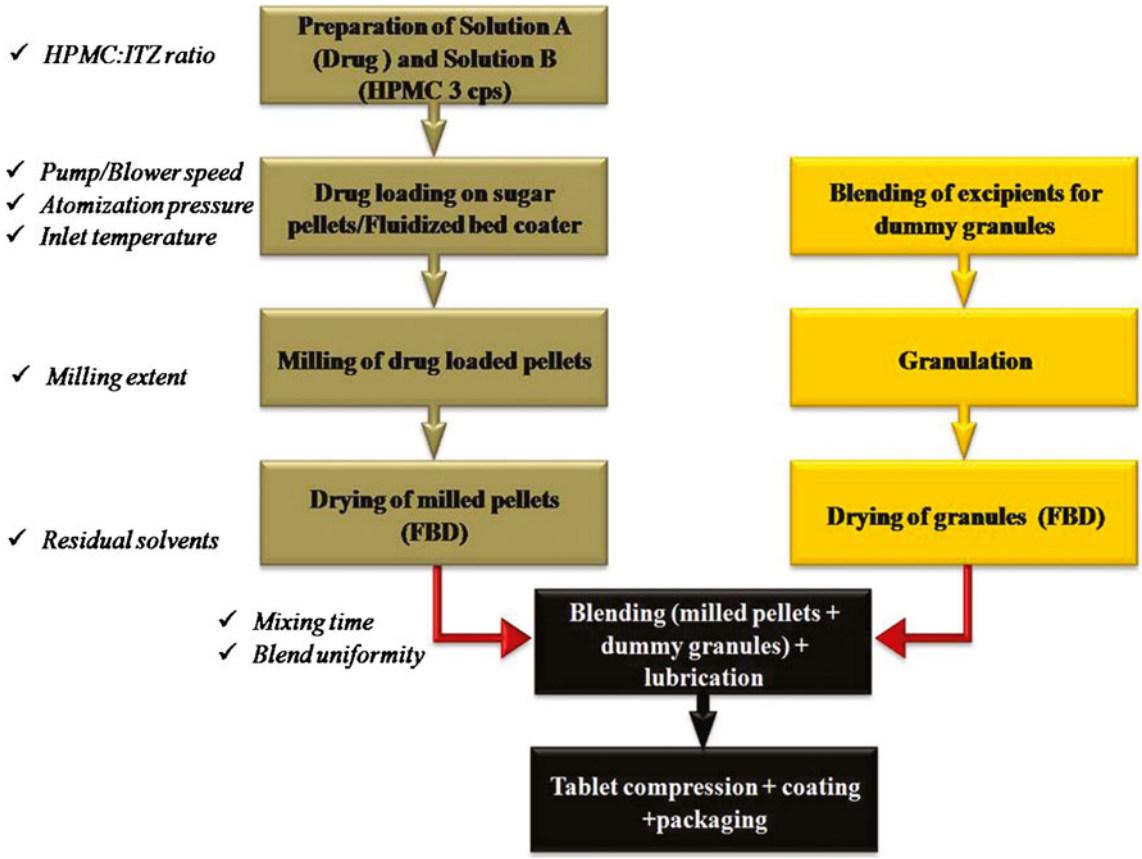

Fig. 1. Sequential processing stages and respective formulation variables involved in product development. Critical process parameters are indicated in *italics outside the box* and *highlighted* with a *tick mark sign*, while the process steps are indicated using *boxes*

and colloidal anhydrous silica were added and blended for 20 min. Tablets were compressed on a Cadmac (India) 18 station rotary tablet press (19.0×9.0 mm, caplet-shaped tablets). Tablets were finally coated with Opadry Pink II.

In Vitro Characterization

Disordered drug delivery system of ITZ was thoroughly characterized at three critical stages, namely formulation of glass system, coating of glass system on sugar pellets and after compression of coated pellets to tablets. Results for the same were collectively summarized in order to get a comparative view of three critical stages and proper elucidation of the mechanistic approaches of solubilization. The samples analysed include pure ITZ, glass system, coated pellets, compressed tablets and physical mixtures of respective steps. All the dose strengths of itraconazole are 100 mg.

Solid-State Characterization

Characterization of solid-state properties of drug incorporated in pharmaceutical dosage form is necessary. Solid-state properties of drug refer to its polymorphic or pseudo-polymorphic form which ultimately defines its *in vitro*/*in vivo* performance. Complete molecular embedment of ITZ in disordered system was confirmed with differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), Fourier transform infrared attenuated transmittance reflectance (FTIR-ATR) Raman spectroscopy (RS) and scanning electron microscopy (SEM) studies.

DSC studies were performed using differential scanning calorimeter (Perkin Elmer, USA). Samples from all the three stages (glass system, coated MUPS and compressed tables) along with pure ITZ, plain pellets, physical mixtures of glass system and MUPS were individually sealed in a 40-μl aluminium pans. An identical empty pan was used as a reference. The samples were scanned at 10°C/min from 20°C to 220°C with a 20-ml/min nitrogen purge. Polymorphic changes in ITZ after molecular embedment in HPMC matrix were evaluated with powder X-ray measurements (Huber Imaging Plate Guinier Camera 670). The samples were scanned from 2θ values of 5° to 60° at an angle of 90°. Solid-state FTIR-ATR studies were performed (Perkin Elmer 2000, USA) between 4,000 and 600 cm^{-1}. Powder samples were placed as such in the sample holder, and the distance between the screw and crystal was adjusted to obtain cumulative results of 16 scans after background correction. RS studies of samples were carried out using Raman system R-3000 (Jasco, Japan) with a 10-cm^{-1} resolution, laser wavelength of 785 nm and a range of 200–2,400 cm^{-1}.

SEM studies were done to evaluate surface topology of physical mixture of glass system components, glass system, coated pellets and pellets of reference product using scanning electron microscope (Joel, Japan). Prior to imaging, mounted powder samples were sputter-coated with gold.

In Vitro Dissolution Studies

In vitro dissolution studies were carried out in simulated gastric fluid without enzymes (pH 1.2) using USP apparatus II-paddle, with a volume of 900 ml and 100 rpm speed. Temperature was maintained at 37±0.5°C. All the formulations were optimized in comparison to the *in vitro* dissolution profile of Sporanox® (100 mg of itraconazole). Ten millilitres of sample was withdrawn, filtered using a 0.45-μm filter and was diluted suitably (2 ml of the sample to 10 ml) to quantify by UV spectroscopy (V-530, Jasco, Japan) at 255 nm. The concentration was determined by a triplicate standard measurement at the same concentration.

Disordered Drug Delivery System at Scale-up

Three batches (viz. EA 9001, EA 9002 and EA 9003), each of 50,000 tablets, were taken to assess the scalability potential of the technology developed in the lab. Blend weight per batch was 46.15 kg. The processing steps were validated appropriately using an in-house developed protocol (KH/MV/01/00/08). Controls for various critical steps were set on the basis of lab-scale experiments.

Equipment parameters were set taking into consideration the previous trials with various product parameters and process parameters. The coating parameters varied drastically as compared to the lab-scale model as the machine dimensions were very different. Drying was tried at various temperatures ranging from 40°C to 80°C using Wurster coater (110 lit. Capacity, Anish Pharma, India). All the process parameters were validated, and batch-to-batch reproducibility with respect to assay, related substances and *in vitro* dissolution profile was evaluated. Various sampling points during drug layer coating process and blending of coated pellets with dummy granules were decided based on the unit operation and the criticality of the step. Figure 2a, b gives a schematic representation of sampling plan from a Wurster coater and sampling plan from a CONTA blender for process validation, respectively.

Tableting parameters were monitored throughout the process with regards to uniformity of mass, average mass, hardness, thickness, friability, disintegration time, *in vitro* dissolution behaviour, assay and related substance. Residual solvent analysis was carried out with validated headspace gas chromatographic method (Varian CP 3800) equipped with a flame ionization detector. Carrier gas used was nitrogen with a flow rate of 4 psi. A thermo-TR VI 30-m×0.53-mm×3-μ film column was used for the purpose. Injector temperature was set at 140°C with the flame ionization detector at 250°C. Injection volume was kept at 900 μl of the head space. Final packaging of tablets was done in Alu-Alu blister pack.

PROCESS VALIDATION—STATISTICAL APPROACH

The tableting process was validated using a statistical tool or a constant called as process capability index (CpK) (25). It was used to measure the robustness of the method. It was measured by using the following formula:

$$\mathrm{CpK} = \frac{\text{Upper limit of specification} - \text{mean}}{3 \times \text{standard deviation}}$$

or

$$\mathrm{CpK} = \frac{\text{Mean} - \text{Lower limit of specification}}{3 \times \text{standard deviation}}$$

Table I summarizes the significance of CpK value in the process scale-up. CpK was found pertaining to parameters like assay, thickness, hardness, disintegration time, friability and uniformity of weight which eventually determined the process suitability. All the parameters were calculated at three stages viz. end of the compression, middle of the compression and start of the compression. The parameters were calculated for all the three batches and eventually summarized.

Stability Studies

The scaled up batches were subjected to stability studies as per ICH guidelines and timely evaluated for ITZ stability and product performance.

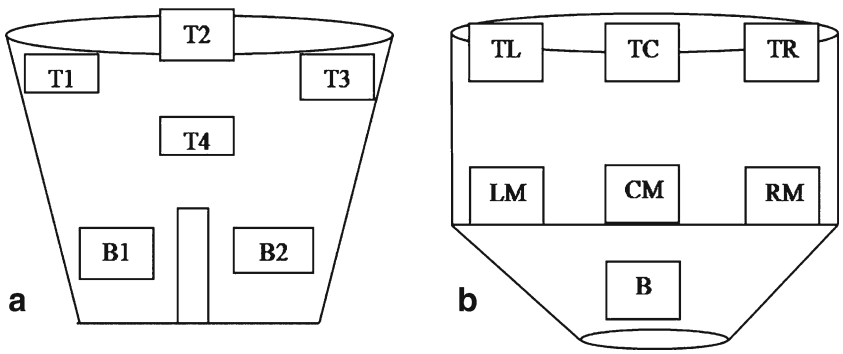

Fig. 2. Sampling plan for process validation of fluid bed coater (**a**) (location *T* refers to *top* and location *B* refers to *bottom*) and sampling plan from a CONTA blender (**b**) (location TL, TC, TR, LM, CM, RM and B refers to *top left*, *top centre*, *top right*, *left middle*, *centre middle*, *right middle* and *bottom*, respectively). A sampler with a dosing adjustment was used for the sample collection (performed by a trained quality assurance personnel)

Table I. CpK Values for Process Validation

CpK value	Significance
Less than 0.8	The process is not capable. Further work needed to develop a more robust process
0.9–1.0	marginal process robustness
1.0–1.25	Satisfactory process robustness
1.25–1.50	Good process robustness
More than 1.50	Excellent process robustness

$$\text{Formula: } \text{CpK} = \frac{\text{Upper limit of specification} - \text{mean}}{3 \times \text{standard deviation}} \text{ or } \text{CpK} = \frac{\text{Mean} - \text{Lower limit of specification}}{3 \times \text{standard deviation}}$$

Bio-equivalence Studies

Study Design

An open label, balanced, randomized, two-treatment, two-period, two-sequence, crossover bioequivalence study of single dose of two ITZ tablets 100 mg (each tablet contains itraconazole 100 mg) and two Orungal capsules 100 mg of Janssen Pharmaceutica (brand equivalent of Sporanox) in healthy human adult male subjects under fed condition was carried out as per the approved protocol. The studies were conducted in accordance with pertinent requirements of the ICH Guidelines for Good Clinical Practice 21 CFR part 320, ICMR (Indian Council of Medical Research) guidelines and National Laws & Regulations. Bio-equivalence studies were initiated with seven healthy human adult male subjects and later on subjects were gradually increased to 17 and finally 24. This was done to understand the inter-subject variability in pharmacokinetic parameters associated with ITZ on oral administration.

Drug Administration

In each of the two study periods, a single dose of test or reference formulation was orally administered with 240 ml of water in the morning 30 min after a standard breakfast. Alternate treatment was administered to the subjects in subsequent periods as per the randomization schedule such that all the subjects should receive both the treatments at the end of the study.

Sample Withdrawal

A total of 26 blood samples were collected during the study. The pre-dose blood sample (2×3 ml) was collected within 1.5 h prior to dosing in each period. The post-dose blood samples (1×3 ml each) were collected at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0 and 72.0 h. The pre-dose and post-dose samples up to 12 h were collected via an in-dwelling intravenous cannula/scalp vein set placed in a forearm vein of the subjects. After 12 h post-dose, samples were taken directly by vein puncture. The ambulatory samples were withdrawn by fresh vein puncture using a disposable sterile syringes and a needle at each time of collection. Blood samples were collected into pre-labelled K2 EDTA vacutainers, and separated plasma was transferred into pre-labelled polypropylene tubes as single aliquot. The ITZ content was determined with validated LC/MS/MS (Perkin Elmer LC pump, Perkin Elmer Auto sampler) method using Hypurity Advance 50×4.6 mm, 5 μm column (Thermo, India).

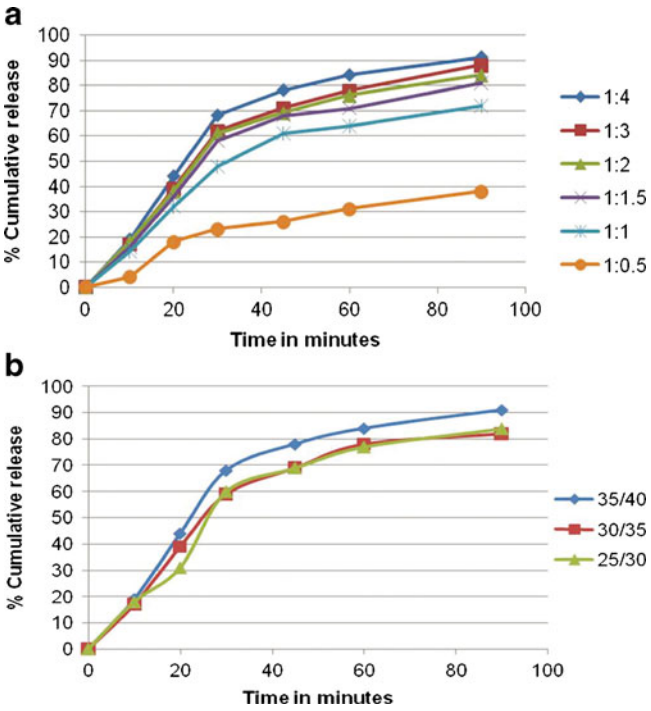

Fig. 3. Comparative dissolution profiles for optimization of drug/HPMC ratio (**a**) and pellet dimension (**b**). The weight of pellets was always maintained constant in the process. Dissolution was performed by using an equivalent of 100 mg of itraconazole

Pharmacokinetic Parameters and Statistical Analysis

Pharmacokinetic parameters like C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el}, $t_{½}$ and $AUC_{0-t}/AUC_{0-\infty}$ were calculated with

Table II. Effect of ITZ/HPMC (Pharmacoat603®) Ratio

ITZ/Pharmacoat 603®	$T_{90\%}$ (min)
1:0.5	180
1:1	145
1:2	75
1:3	47
1:3.5	45
1:4	30
1:5	85

ITZ itraconazole, $T_{90\%}$ time required to release 90% of drug

Table III. Optimization of Amount of Pellets

Core pellets (mg/dose)	Drug (mg)	HPMC (3 cps) (mg)	$T_{90\%}$ (min)
500	100	150	30
450	100	150	35
400	100	150	48
350	100	150	60
300	100	150	80

HPMC hydroxypropyl methyl cellulose, $T_{90\%}$ time required to release 90% of drug

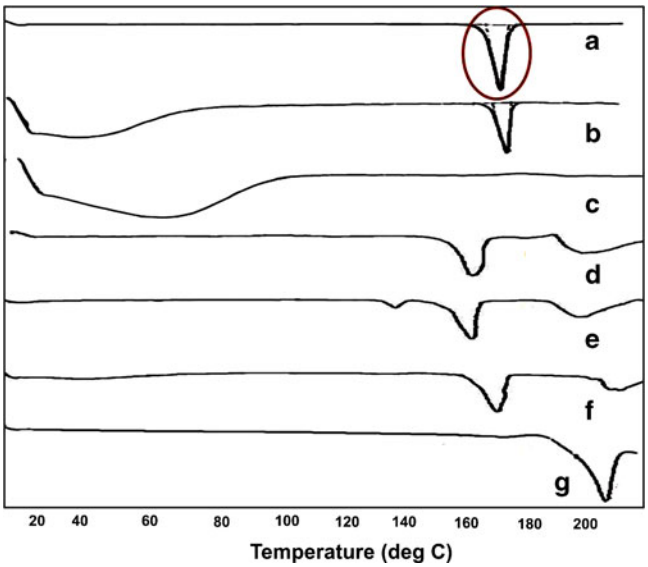

Fig. 4. DSC studies, *A* ITZ, B physical mixture for glass system, *C* glass system, *D* plain pellets, *E* physical mixture for pellets, *F* MUPS and *G* tablets. The peak for ITZ is seen only in drug and the PM, while it disappears in all other formulations indicating a molecular interaction and formation of disordered system

arithmetic means, standard deviations and coefficients of variation using non-compartmental model of WinNonlin Version 5.2. Additionally, geometric means were calculated for $AUC_{0-\infty}$ and C_{max}. The log-transformed pharmacokinetic parameters (C_{max} and $AUC_{0-\infty}$) were analyzed using a general linear model analysis of variance (ANOVA) model with the main effects of treatment. The main effects tested were at the 0.05 level of significance against the residual error (mean square error) from the ANOVA as the error term.

RESULTS

Disordered Delivery System of ITZ at Lab Scale

In case of glass system in the form of film, it was observed that a transparent film was formed when the proportion of ITZ/HPMC was varied from 1:0.5 to 1:4. As the amount of polymer was increased, the drug release also increased proportionately, but after a certain level (1:4), increasing the polymer amount led to decrease in drug release (Table II).

However, when the same glass system was loaded onto sugar pellets, ITZ/HPMC ratio of from 1.0:1.5 was found to be optimum for MUPS as lower ratios gave less drug release and even higher ratios retarded the release to some extent (Fig. 3a).

Optimization of pellet dimension was done with $T_{90\%}$ (time required for 90% release of ITZ) measurements of the drug from the formulations. It was seen that as the size of the pellets decreases, the $T_{90\%}$ also decreases, i.e. a faster release is achieved. Pellet of dimension 35/40 mesh ASTM gave the most desired release as evident from Fig. 3b.

It was found that as the amount of pellets in each unit formulation increased, the dissolution rate also increased (Table III). The objective, however, was to achieve a release close to the innovator product; thus, a drug loading with 350 mg pellets/unit formulation was considered to be optimum which further gave a similarity factor of above 82.

In a Wurster air suspension, the process parameters play a very important role in determining the desired success and reproducibility. A fine balance must be struck between the blower speed, coating rate, air pressure and the bed temperature. All the efforts must be directed towards maintaining a constant product bed temperature. It was seen that as the bed temperature was increased above 41°C, the nozzle was clogged. Thus, a temperature of around 38±2°C was considered to be optimum, and a lower temperature led to formation of agglomerates owing to insufficient drying of the pellets. Atomization pressure of around 1.5±0.5 kg/cm^2 and peristaltic pump speed of about 5–8 rpm were considered optimum for coating process.

In Vitro Characterization

Solid-State Characterization

From the results of DSC studies as depicted in Fig. 4, it is clear that ITZ endotherm is completely masked after molecular dispersion inside HPMC-3 cps (Phamacoat 603®). PXRD studies of glass system showed that as the drug was

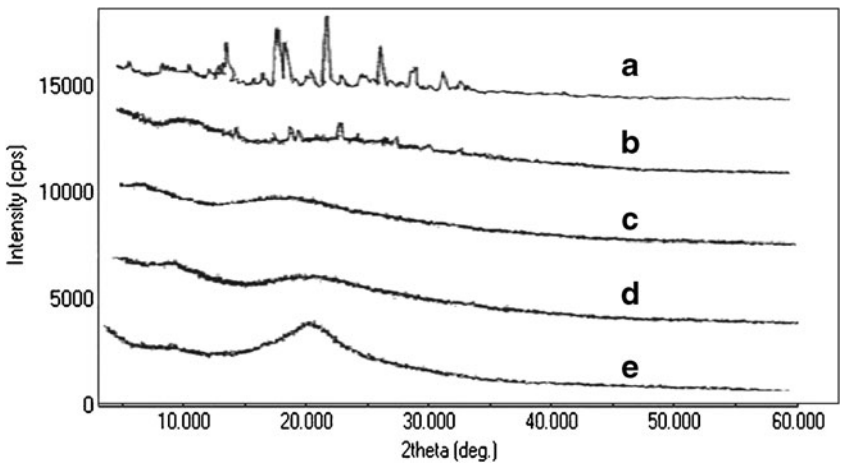

Fig. 5. X-ray diffraction studies, *A* ITZ, *B* physical mixture (ITZ + excipients involved), *C* glass system, *D* MUPS and *E* tablet. Disappearance of critical peaks of itraconazole symbolizes formation of disordered system

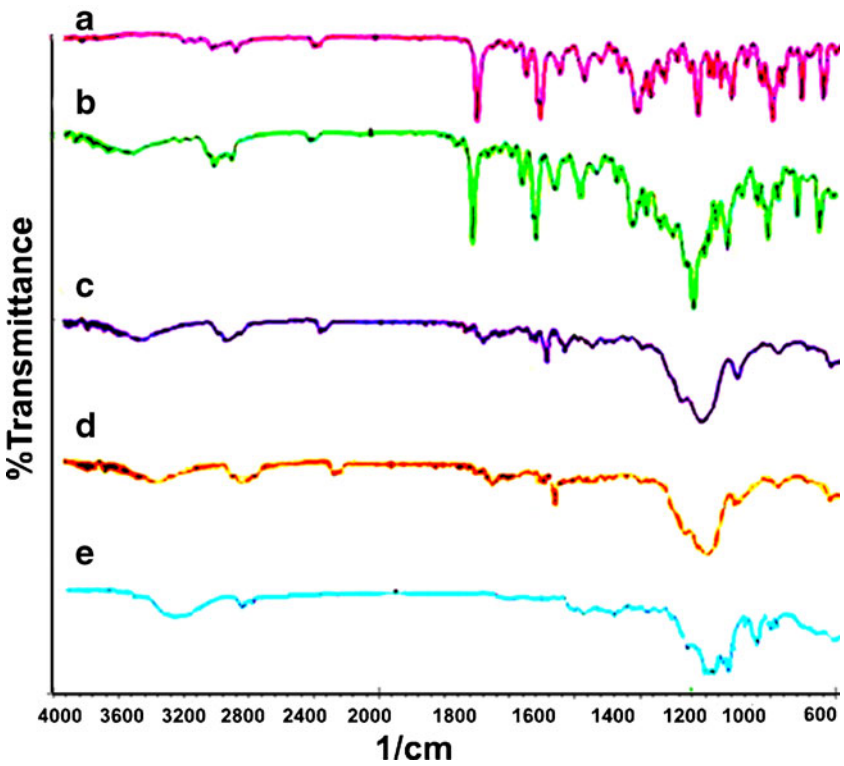

Fig. 6. FTIR-ATR studies, *A* ITZ, *B* physical mixture for glass system, *C* glass system, *D* MUPS and *E* tablets

molecularly dispersed inside the polymer matrix, the original crystallinity was reduced to an extent of it becoming almost an amorphous compound (Fig. 5).

FTIR studies of glass system of ITZ further confirmed the level and position of molecular interaction of HPMC with ITZ (Fig. 6). Characteristic peaks associated with aromatic ring of

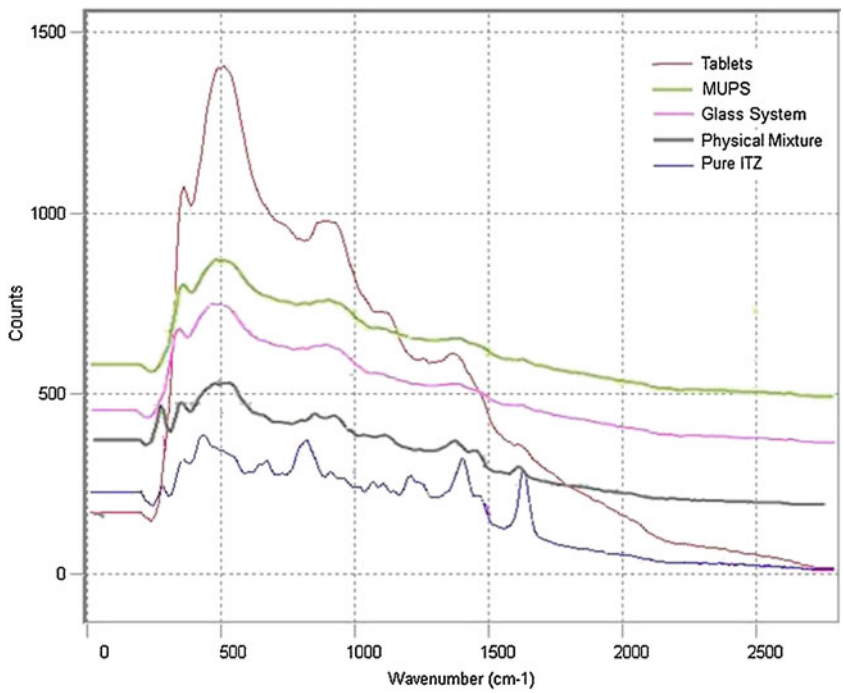

Fig. 7. Raman spectroscopy studies of tablets, MUPS, glass system, PM and pure ITZ indicated by *different colours*

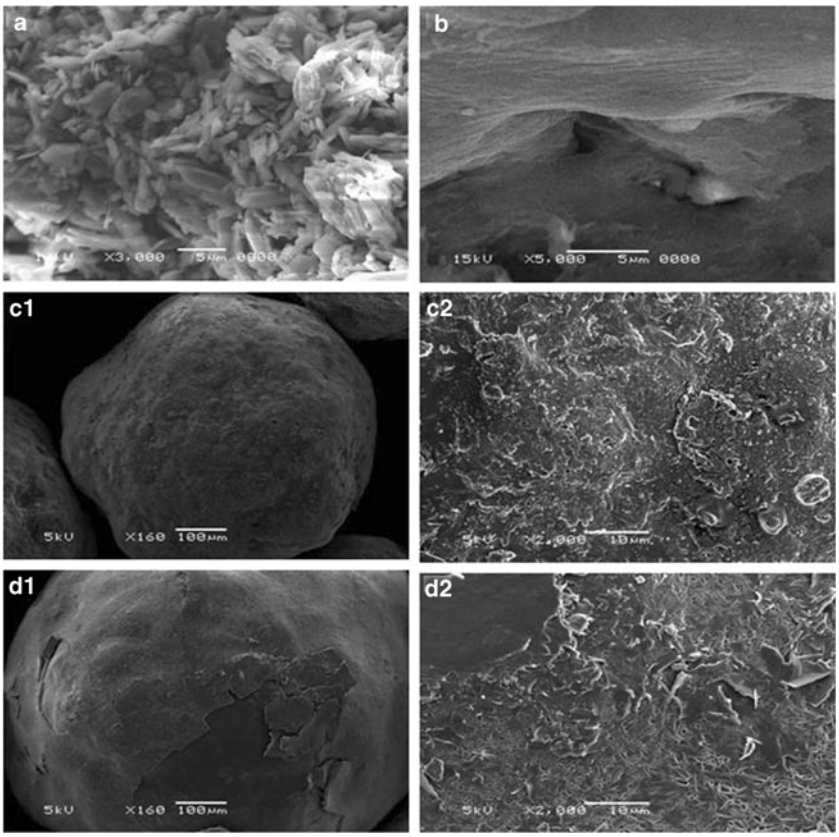

Fig. 8. SEM studies, *A* ITZ + excipients involved, *B* glass system, *C1* and *C2* MUPS, *D1* and *D2* Sporanox

ITZ, straight chain alkyls and the C=O bond were selectively masked with significant reduction in peak intensity. ITZ glass film loaded onto MUPS and compressed into tablets exhibited no change in molecular embedment of ITZ inside HPMC matrix. Raman spectroscopic studies (Fig. 7) of ITZ disordered system at three different stages were in conjunction with the results of DSC, PXRD and FTIR studies.

SEM images (Fig. 8) also support the results observed with other solid-state characterization tools employed. In case of glass system, there is a single-phase smooth surface seen as compared to a crystalline two-phase system in physical mixture in which the crystals of ITZ are clearly evident. Developed MUPS surface was found to be smooth uniformly coated single-phase system as innovator product Sporanox. Absence of drug crystals on the surface of the pellets further supported the true molecular encapsulation phenomenon.

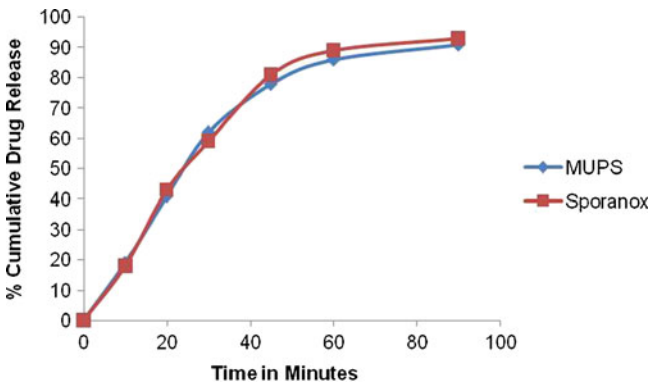

Fig. 9. *In vitro* dissolution studies of the developed tablet formulation vis-à-vis Innovator Sporanox

In Vitro Dissolution Studies

The dissolution was compared to the innovator formulation. It was found that the *in vitro* drug release from the

Table IV. Validation of Fluid Bed Coating of Pellets

	% Assay		
Sample point	EA 9001	EA 9002	EA 9003
Location—T_1	106.89	121.12	104.85
Location—T_2	102.01	117.15	103.01
Location—T_3	103.5	119.0	105.32
Location—T_4	103.05	118.14	103.82
Location—B_1	110.2	117.24	105.75
Location—B_2	106.98	123.03	106.42
Composite	105.84	118.81	106.46
Average	105.49	119.21	105.09
Minimum	102.01	117.15	103.01
Maximum	110.2	123.03	106.46
SD	2.84	2.15	1.30
%CV	2.7	1.8	1.23

SD standard deviation, *% CV* % coefficient of variation

Table V. Validation of Blending Step

Sample point	% Assay (before lubrication)			% Assay(after lubrication)		
	EA 9001	EA 9002	EA 9003	EA 9001	EA 9002	EA 9003
Location 1—TL	101.37	102.22	102.22	102.22	102.8	105.53
Location 2—TC	99.0	102.01	102.01	102.01	101.4	107.61
Location 3—TR	101.91	103.57	103.57	103.57	100.3	103.82
Location 4—ML	99.31	102.5	102.5	102.5	99.1	105.99
Location 5—MC	100.55	103.23	103.23	103.23	101.7	109.28
Location 6—MR	101.92	101.99	101.99	101.99	99.9	106.21
Location 7—B	98.91	102.86	102.86	102.86	99.4	107.08
Composite	99.48	102.51	102.51	102.51	100.3	104.1
Average	100.31	102.61	102.61	102.61	100.61	106.20
Minimum	98.91	101.99	101.99	101.99	99.10	103.82
Maximum	101.92	103.57	103.57	103.57	102.80	109.28
SD	1.29	0.57	0.57	0.57	1.26	1.81
%CV	1.29	0.56	0.56	0.56	1.25	1.70

TL top left, *TC* top centre, *TR* top right, *ML* middle left, *MC* middle centre, *MR* middle right, *B* bottom, *SD* standard deviation, *% CV* % coefficient of variation

MUPS was comparable with that of the innovator formulation giving a similarity factor of 82.73. The dissolution profiles were almost super-imposable (Fig. 9).

Disordered Drug Delivery System at Scale-up

Formulations were scaled up at 50,000 tablets/batch in three separate batches successfully with an extensive validation of all the process parameters.

Critical Scale-up Considerations

The coating parameters were redesigned as per the process requirement and validated in detail to ensure product reproducibility. Briefly in pellet coating process, the peristaltic pump speeds were increased up to 40 rpm. Atomization speed was simultaneously increased up to 2.2 kg/cm^2. The bed temperature was almost the same. Table IV gives a detailed validation data of fluid bed coating of pellets. This modification led to a very rapid process speed and thus proved to be cost-effective. Also a relatively safer class III solvent, isopropyl alcohol, was used in major proportions.

It was observed that friability of tablets compressed with unmilled pellets was relatively high. Compression of milled pellets was found to resolve the problem of high friability but failed with the aesthetic appearance. Milling of pellets before compression and addition of dummy granules resulted in better compressibility with desired cushioning effect which further improved the aesthetic appeal. Milling of pellets before compression was also proved to be beneficial for controlling residual solvent content. The final optimized MUPS and tablet formula is presented in Tables VII, VIII and IX of "Appendix 1", respectively.

Milled pellets were uniformly blended with dummy granules. The content of ITZ sampled from different points was within the in-house set limits of 100–120% (*w*/*w*). Validation of blending step was performed before and after lubrication (Table V).

Process Validation—Statistical Approach

The process capability index was the main parameter determining the scale-up success of the formulation. Three scale batches, namely EA 9001, EA 9002 and EA 9003, were studied with regards to the CpK or process capability index (Fig. 10).

The batches EA 9001, EA 9002 and EA 9003 exhibited residual isopropyl alcohol content as less than 4,000 ppm. It was found that in all the three batches, the total impurities were below 0.6% and any individual impurity was below 0.3% (*w*/*w*). Where in the limits set were: total impurity not more than 1.25% and any single impurity not more than 0.5%.

In Vitro Drug Release Studies on Scale-up Batches

Figure 11 gives a dissolution profiling of the three scaled up batches v/s Sporanox®. It could be seen that they are almost super-imposed on each other with a similarity factor of over 70.

Stability Studies

The assay (90% to 110% of labelled amount) and content uniformity (85% to 115% of labelled amount) of tablets were within the specified limits. Total (<1.5%) and individual impurities (<0.5%) were found under control. There was no change in physical appearance of product. *In vitro* release of ITZ on stability was always comparable with initial release profile (>70% in 90 min). Developed disordered delivery system of ITZ in the form of tablets was found to be stable up to 12 months at real time and accelerated conditions without any significant alteration in product characteristics and performance.

Bio-equivalence Studies

Bioequivalence studies of developed ITZ tablets (test) and Orungal capsules (reference) were successfully done in

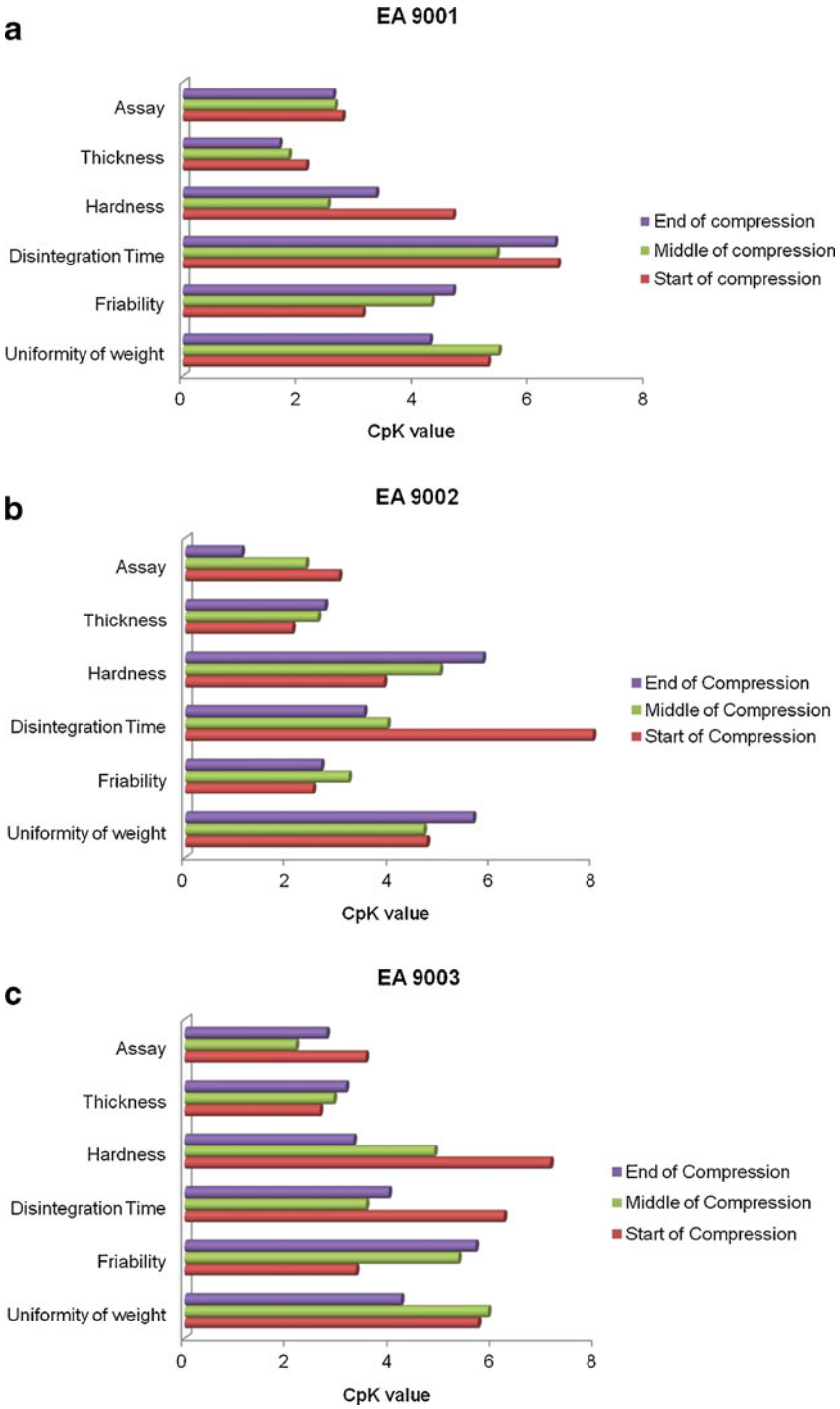

Fig. 10. Process validation, **a** EA 9001, **b** EA 9002 and **c** EA 9003

24 healthy volunteers at fed state. The mean values for pharmacokinetic variables are shown in Table VI. Figure 12a shows the mean concentration–time curve for both formulations of ITZ in seven subjects. Peak plasma levels were obtained near to 4 h for the test and reference formulations. Gradual increase in subjects from seven to 17 exhibited alterations in pharmacokinetic parameters. Figure 12b shows the mean concentration–time curve for both formulations of ITZ. On further increase in subjects up to 24, it was observed that both reference and test formulations show variations in pharmacokinetic parameters. Figure 12c shows the mean concentration–time curve for both formulations of ITZ. It was observed that ITZ levels in plasma for test formulation were declined as compared to reference formulation with increase in number of subjects.

DISCUSSIONS

Lab-scale development with critical evaluation of process parameters allowed us to understand the fundamentals of glass system formation, effect of ITZ/HPMC ratios on dissolution profile, effect of pellet size and amount on process

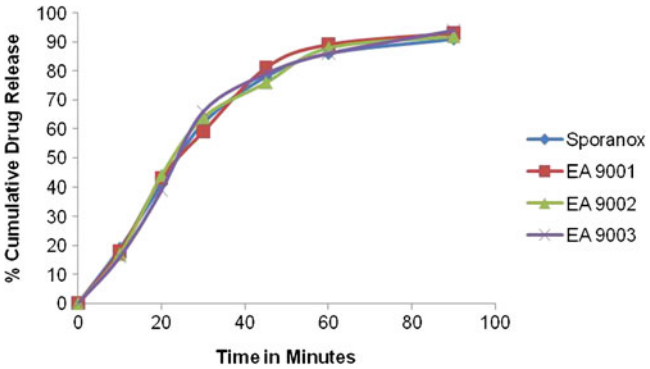

Fig. 11. Dissolution profile of the scaled up batches v/s reference product

performance and product characteristics. Increasing the amount of polymer after a certain extent seemed to have a negative impact on the dissolution characteristics of the formulation. After a concentration ratio of 1:4 (drug/polymer), the release was hampered. This could be due to formation of a thick matrix of HPMC. Albeit soluble, at higher concentration, soluble matrices can also hinder drug release. We found that there is a fine balance between drug release, surface area and film uniformity. With a pellet dimension greater than that of 35/40 mesh, the drug release was lower than desired, due to the large surface area, and above 35/40, we could not coat uniformly due to formation of doubles and triplets, which become very hard after drying and hinders the drug release. This is again due to the fact that there is an increase in the surface area thereby leading to greater free energy of the system and thus higher solubility/dissolution rate. Ease of coating was also considered one of the parameters for the optimization of the core pellet dimension. When the size of the pellet decreased, agglomeration was seen; thus, a size beyond 35/40 mesh ASTM was not considered for optimization as agglomeration was predominant in those cases. Lactose granules were fragile in nature and besides had irregular surface leading to non-uniform coating and thus less surface area for dissolution. Increased amount of pellets would also mean a greater hydrophilic component in the system, which in turn may help improve the drug release. Also there is an increased effective surface area with increasing amounts of the pellets. As more cores were available, the total drug loaded on each pellet was less, thereby leading to overall increase in the surface contact points and thus wettability. In a Wurster air suspension, the process parameters play a very important role in determining the desired success and reproducibility. A fine balance must be struck between the blower speed, coating rate, air pressure and the bed temperature. All the efforts must be directed towards maintaining a constant product bed temperature. Any change above 3°C was found to be detrimental to the success of the process, and essentially every failure in process resulted in hampered drug dissolution. Thus, maintaining the process parameters form the heart of the formulation design.

In-depth solid-state characterization of developed formulation at three critically important stages, namely formation of glass system, coating of glass system onto pellets and compression of crushed pellets into tablets, further helped in better understanding of mechanistic approach involved in ITZ solubilization and stabilization (21–24,26). DSC is an important test for molecular embedment of drugs inside polymer matrix. If some form of the drug was not embedded, then it would have been evident from appearance of an endotherm. In this case, however, molecular dispersion is confirmed. Same was the case with XRD. We could mask important peaks of itraconazole with 2θ of 14.04°, 14.38°, 17.42°, 17.88° and 23.40° in our formulations. All peaks, except the one at 23.40°, were absent in tablet formulation but present in physical mixture and itraconazole, indicating a molecular embedment of the drug (shown in "Appendix 2"). In case of FTIR, characteristic peaks associated with aromatic ring of ITZ, straight chain alkyls and the C=O bond were selectively masked with significant reduction in peak intensity indicating molecular encapsulation of the drug. Same was the case with Raman spectroscopy. Being more sensitive to molecular level changes in drug relaxation tendencies, we used Raman spectroscopy to gain a clearer understanding of the interactions. SEM studies also supported the results observed with other solid-state characterization tools employed, as we could see a uniform blend in case of coated pellets as opposed to a hybrid blend in case of drug–polymer physical mixture. The disintegration rate of the tablets was adjusted in such a way that it was overlapped with the capsule core dissolution and subsequent wetting of the innovator pellets. This was done by optimizing the hardness and friability of the uncoated tablets.

The process parameters set up at lab-scale development need to be marginally modified at scale-up level. The coating process was primarily affected with scale-up from lab scale to production scale owing to the larger size of the instrument. The weight gain of pellets and loss on drying were considered as the main parameters for optimization of these parameters at a scale-up level. Process validation was done by using CpK or process capability index. The greater the value, the better is

Table VI. Pharmacokinetic Parameters: Bio-equivalence Studies

Parameter	Test	Reference	Test	Reference	Test	Reference
Number of volunteers	7	7	17	17	24	24
C_{max} (ng/ml)	150.8±49.34	171.72±68.21	192.2±93.17	219.55±81.56	181.98±99.47	229.37±86.46
AUC last (hng/ml)	2,236.9±389.58	2,179.3±477.29	2,899.3±672.37	3,005.1±589.28	2,684.5±789.65	3,196.2±857.86
AUC infinity (hng/ml)	2,553.4±425.16	2,366.2±269.18	3,179.7±659.78	3,331.8±712.94	2,949.9±643.87	3,525.7±769.96
CI 90 (AUC last)	93.56–112.61		81.9–113.66		70.7–99.78	
CI 90 (AUC infinity)	95.21–122.31		79.57–114.46		70.22–99.70	
Ratio (% ref) AUC last	102.65		96.48		83.99	
Ratio (% ref) AUC ∞	107.91		95.43		83.67	

AUC area under the concentration–time curve, *CI* confidence interval

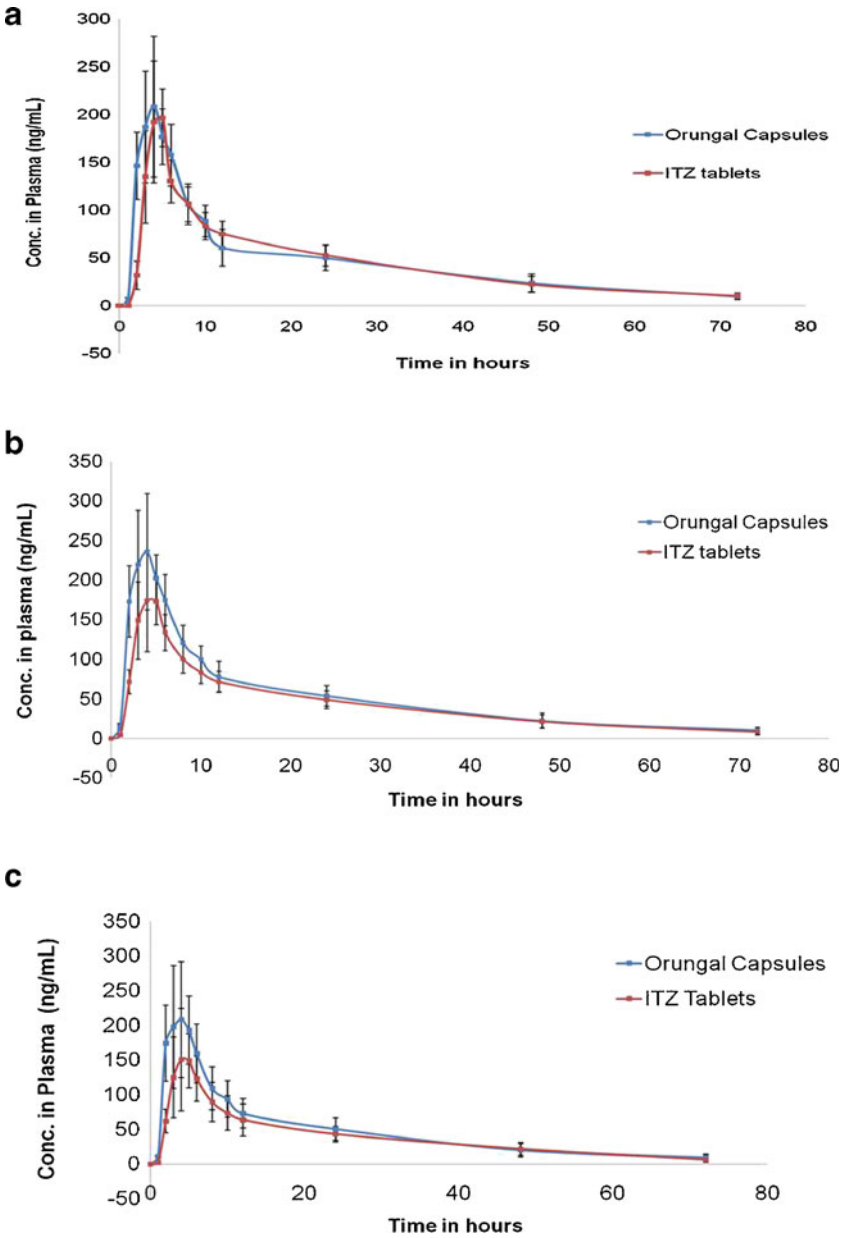

Fig. 12. Bioequivalence studies **a** in seven volunteers, **b** in 17 volunteers and **c** in 24 volunteers

the process. We found that our process was very robust with high CpK values in critical process steps. Any value above 1.5 is normally considered as an excellent value. In our case, we obtained values greater than 1.5 in all process steps indicating a robust process. Keeping the organic solvent level below the FDA norms is an important step in any formulation design. With our novel milling–drying method using the Wurster coater–dryer, we were able to achieve this objective successfully. We have summarized the saturation solubility results of itraconazole at various formulation steps in "Appendix 3". There is significant enhancement of drug solubility after molecular embedment in HPMC (3 cps). We could also determine that the formulas developed were stable based on the assay, content uniformity, related substance measurement, *in vitro* drug release as per ICH guidelines in three conditions of temperature and humidity (25°C, 60% relative humidity (RH), 30°C, 65% RH and 40°C and 75% RH). DSC results of the formulations after 6 months at 40°C and 75% RH ascertained the stable amorphous behaviour of itraconazole in all formulations ("Appendix 4").

Considering a great deal of similarity in the *in vitro* dissolution behaviours in simulated gastric fluid without enzymes as per US FDA recommendation, we were confident about the bioequivalence of the formulation with the innovator. However, an anomalous behaviour of the tablets was seen *in vivo*. The equivalence behaviour changed with the increase in number of subjects. The tablet formulation was bioequivalent to Sporanox when the study was performed in seven and 17 subjects, but the bioequivalence pattern changed on increasing the subject count to 24. From the literature, we clearly understand

that the pharmacokinetics of orally administered itraconazole in humans are characterized by considerable inter-individual variation in drug absorption, extensive tissue distribution, with the concentrations in tissue being many times higher than those in plasma, and an elimination half-life of ca. 24 h (27–29). We hypothesize that this bio-variable nature of itraconazole may be partly responsible for the observed anomalous behaviour. Additionally, the pharmacokinetics of orally administered itraconazole in plasma are dose dependent, and absorption from the gastrointestinal tract is affected by various factors, such as food intake *versus* fasting state (30,31), gastric pH (32,33), drug interactions (33,34), AIDS and the pharmaceutical formulation of the drug (35,36). As per our power calculations, we found that the % CV in the subjects for the innovator product was in excess of 35%, and as per the reported statistical calculations, the number of patients must be more than 85. However, in our study, we have used a pilot scale of 24 subjects. Itraconazole is extensively metabolized in humans, yielding over 30 metabolites, including the antifungally active metabolite hydroxyitraconazole. When itraconazole in plasma is measured by a bioassay, values reported are approximately 3.3 times higher than those obtained by HPLC due to the presence of the bioactive metabolite, hydroxyitraconazole, that can be found in concentrations ~2× that of the parent drug, which suggests a major contribution to the overall mycological activity observed during itraconazole therapy (29). The FDA recommendations for itraconazole BE study states that the measurement of both the moieties has to be done in a clinical study. However, considering the current scope of our study, we did not measure the plasma levels of the active metabolite and strongly believe that results from such studies would strongly help support our data. We also strongly feel that in cases where molecules like itraconazole are studied, it is of paramount importance to study the *in vitro* release behaviour in multiple bio-relevant media. We would also like to elucidate that in generic drug product development, usage of excipients similar in nature to the innovator product could prove useful.

CONCLUSION

Itraconazole, a recalcitrant molecule with poor dissolution in biological conditions, was successfully rendered amorphous by molecular embedment in a simple, readily available low viscosity polymer viz. HPMC (3 cps) was converted into a tablet formulation using an industrially scalable process. According to our process, the pellet milling step coupled with drying led to a speedy removal of volatile organic solvents. Disordered drug delivery led to a complete embedment of ITZ in matrix of lower viscosity polymer, Pharmacoat®, which further helped in faster removal of residual solvents thereby yielding a cost-effective process. The process used for fabrication of tablets was simple, cost-effective and was validated successfully at scale-up stage. Bioavailability studies highlighted the bio-variable nature of itraconazole, thereby showing a reduced equivalence with increase in number of subjects. We attribute this anomalous behaviour to the bio-variable nature of itraconazole and suggest that metabolite assay if performed would have been useful to understand the mechanistic behaviour of itraconazole *in vivo*. Further studies are in progress and would help us understand the bioequivalence nature from a regulatory stand point. Further studies are also warranted to support the bio-variable nature of itraconazole in such studies.

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Conflict of Interest There are none.

APPENDIX 1: OPTIMIZED FORMULAS

MUPS

Table VII. The Unit Formula per Tablet for Coating of Pellets: A 20% Overage Was Added to Compensate for Process Loss

Sr. No.	Ingredients	g/batch
1	Core pellets (25–30)	350
2	Itraconazole	99.305+19.861 (20% overages)
3	HPMC (3 cps)	150+30 (20% overages)
Sr. No.	Ingredients	Lit/batch
1	DCM (50%)	1.4955
2	IPA (40%)	1.1964
3	Absolute ethanol (99.9%, *v/v*) (10%)	0.299

HPMC hydroxypropyl methyl cellulose, *DCM* dichloromethane, *IPA* isopropyl alcohol

Optimized Unit Tablet Formula

Table VIII. Optimized Unit Tablet Formula

Sr. No.	Ingredients	mg/tab
1	Drug-coated pellets (milled/40#)	562.0776[a]
2	Lactose MCC granules (25#)	280.9224[b]
3	Sodium starch glycholate	30
4	Hydroxypropyl cellulose (HPC LH 11)	30
5	Magnesium stearate	10
6	Aerosil R 972	10

MCC microcrystalline cellulose, *HPC* hydroxypropyl cellulose
[a] Calculated based on the assay values
[b] Amount calculated by using the formula: 843 mg (optimized weight of milled pellets and dummy granules)−amount of milled pellets

Table IX. Unit Formula for the Preparation of Dummy Granules

Sr. No.	Ingredients	mg/tab
1	Lactose 200 M	108
2	MCC PH 101	72
3	Ac-Di-Sol	45
4	PVP K 29/32	18

MCC microcrystalline cellulose, *PVP* polyvinyl pyrrolidone

APPENDIX 2: PEAK ASSIGNMENT IN XRPD

Table X. Peak Assignment in XRPD

	Areas			RDC	
2θ (°)	ITZ	PM	Tablets	PM	Tablets
14.04	7,421	1,114	–	0.150	0
14.38	8,986	1,167	–	0.129	0
17.42	6,209	1,583	–	0.254	0
17.88	6,042	1,071	–	0.177	0
23.40	2,893	1,669	1,096	0.576	0.378

ITZ Itraconazole, *PM* Physical mixture, *RDC* relative degree of crystallinity

APPENDIX 3: SATURATION SOLUBILITY AND SOLUBILITY ENHANCEMENT FACTOR FOR ITZ IN VARIOUS FORMULATIONS

Table XI. Saturation Solubility and Solubility Enhancement Factor for ITZ in Various Formulations

Type	Saturation solubility (μg/ml)	Enhancement factor
ITZ	6.5	–
PM	8.6	1.32
MUPS	100.35	15.43
Innovator	104.25	16.03

ITZ Itraconazole, *PM* Physical mixture of ITZ with HPMC, *MUPS* Glass system coated on pellets used for tablets, *Innovator* marketed formulation used for comparison

APPENDIX 4: DSC OF ALL FORMULATIONS AFTER 6 MONTHS AT 40°C AND 75% RH

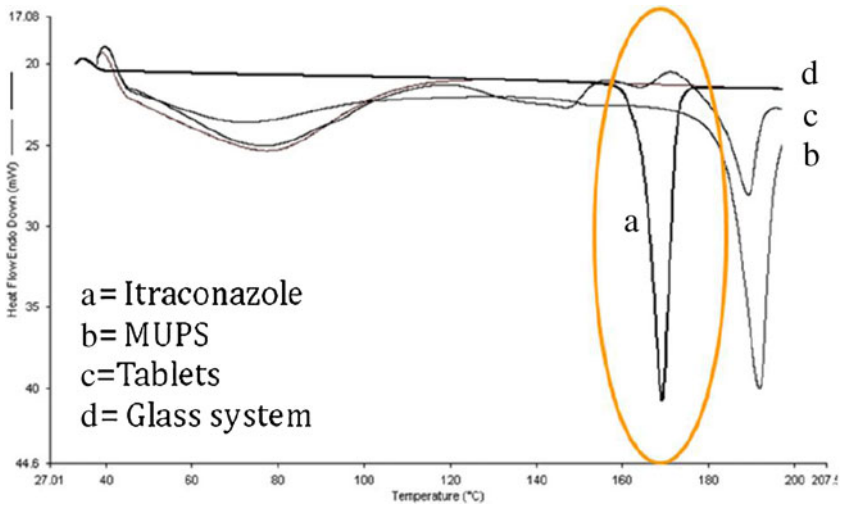

Fig. 13. DSC of all formulations after 6 months at 40°C and 75% RH

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