



## PHARMACOKINETIC BIOAVAILABILITY COMPARATIVE STUDIES OF ITRACONAZOLE SPHERICAL AGGLOMERATED DENSE COMPACTS WITH COMMERCIAL PRODUCT

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### Abstract:

Pharmacokinetic parameters including  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ ,  $C_{max}$ ,  $T_{max}$ ,  $T_{1/2}$  and elimination rate constant ( $K_{el}$ ) were determined from plasma concentrations of Itraconazole spherical agglomerated dense compacts by  $2^4$  factorial design canvassed with a commercially available formulation of Itraconazole. Foremost spherical agglomerates of Itraconazole prepared with PEG 6000 which is hydrophilic polymer and evaluated for different compressibility parameters and invitro dissolution studies, studies indicated that the drug release can be modulated by using hydrophilic polymers and the best one compressed as dense compacts and appraised for pharmacokinetic parameters in rabbit models. Sensitive and selective high performance liquid chromatographic method was used to determine drug plasma concentrations with good linearity in the range of 0.5 to 20 $\mu$ g/mL ( $r^2=0.995$ ,  $n=7$ ). Study ascertained meliorated values of  $AUC_{0-24}$ (15.789  $\pm$ 12.9, 97.334  $\pm$ 7.478).

**Key word:** Pharmacokinetic study, Itraconazole, spherical agglomerates, spherical crystals, rabbit model.

### Introduction:

The conventional tableting method used involves first making granules and then compressing into tablets, but the need in recent years for process validation, GMP and automation of production processes has focused renewal of attention on the direct tableting, which involves few steps <sup>(1)</sup> Itraconazole, is a novel NSAID, selective COX2 inhibiting drug used in the treatment of rheumatoid arthritis and osteoarthritis and to relieve inflammation and pain after surgery <sup>(2)</sup>. Itraconazole exhibits poor flow and compression characteristics and hence Itraconazole selected as suitable candidate for spherical crystallization process to improve flow properties and compressibility. Further, Itraconazole shows poor oral bioavailability due to low aqueous solubility <sup>(3)</sup> hence in such case enhancing bioavailability is a valuable goal to improve therapeutic efficacy. Estimation of Itraconazole in plasma by methods like HPLC<sup>(4)</sup>, Mass spectrometry in human plasma <sup>(5)</sup> was reported.

In The present study intended to prepare spherical crystals of Itraconazole to improve aqueous solubility, dissolution rate and bioavailability besides improving its micromeritic properties using PEG-6000 which is hydrophilic polymer<sup>(6)</sup> the best formulation compressed into dense compacts and in this study further intended to determine the pharmacokinetic parameters for optimized formulation by factorial design and followed by in vivo study, rabbit as an animal model and comparative to a marketed formulation.

## 2. Materials and methods:

### 2.1 Materials:

Itraconazole (99.3% purity), as a gift sample was received from M.S Hetero pharmaceutical, Hyderabad. Acetonitrile and water were HPLC grade from RFCL Ltd (Mumbai, India). bath sonicator was used for degassing (PCI Analytics, Mumbai, India). Elico pH meter was used for adjustment. Ten station rotator tablet punching machine used for compression of tablets with 7 mm flat shaped punch (Elite tablet punching machine, Mumbai.) REMI R-2 research centrifuge and REM-101 cyclo mixer were used for extraction of drug from plasma (REMI Dectrotechnik Ltd., Vasai India).

### 2.2 Formulation of Itraconazole spherical agglomerates.

A solution of Itraconazole (500 mg) in acetone (3 mL) was added to a solution of Poly ethylene glycol 6000 (1-4% w/v) in 100 mL distilled water. The mixture was stirred continuously using digital mechanical stirrer (IKA motors. Mumbai) at 500 rpm, the bridging liquid (Dichloromethane; 0.5 mL) was added drop wise (table 1) and stirring was continued for 30 min. The agglomerates were separated by filtration using whatman filter paper (No.1) and dried for 24 hours at room temperature.

INGREDIENTS	F1	F2	F3	F4
Itraconazole (mg)	500	500	500	500
PEG 6000 (%w/v)	1%	2%	3%	4%
Acetone (mL)	3	3	3	3
DCM (mL)	0.5	0.5	0.5	0.5
Water (mL)	100	100	100	100

Table 1: Formulation of Itraconazole Spherical agglomerates.

### 2.3 Compression of dense compacts

Amid of all spherical agglomerates, the best performed agglomerates in terms of compressibility properties selected as optimized batch. It was thought of interest to study the effect of super disintegrants such as Ac-Di-Sol (CCS), Polyplasdone XL (CP) and Explotab (SSG) on the drug release of Itraconazole.

Spherical crystals were made into dense compacts with 4% w/w CCS, CP and SSG separately using Elite tablet punching machine, 10-station rotary punching machine (7 mm punch and die set) at a compression force of 6 tons.

### 2.4 In vitro dissolution studies

The *in vitro* dissolution studies<sup>(7)</sup> were carried out using 8 station USP XXIII dissolution testing apparatus (Electrolab, Mumbai, India). The dissolution medium used was 900 mL, mixture of phosphate buffer solution pH 6.8 and water (1:1) used as dissolution medium. The agglomerates containing 80 mg of Itraconazole were weighed and then introduced into the dissolution medium. The medium was stirred at 50 rpm using paddle at  $37 \pm 0.5$  °C. The samples were collected, filtered through whatman filter paper (0.45 $\mu$ M) and analyzed spectrophotometrically at 340 nm.

### 3. *In vivo* studies:

#### 3.1 *In vivo* Chromatographic conditions of HPLC<sup>(8)</sup>

The HPLC system included system was automatically processed with and fully automated processor with Elite program. The pre column used in HPLC system was Capcell pak and the main analytical column used in the system was Capcell Pak C18. Mobile phase A ( a 12:88 v/v mixture of Acetonitrile and 10 mM phosphate buffer) and Mobile Phase b ( A 35:65 v/v mixture of Acetonitrile and 10 mM phosphate buffer, PH 6.8) was pumped into the HPLC system at a flow rate of 500  $\mu$ L/min and 90  $\mu$ L/min. The Itraconazole concentrations in effluent of the system were discovered with a UV detector at 340 nm and the temperatures in the system of pre and analytical columns were maintained at 40<sup>0</sup> C and auto sampler chamber was sustained at 4<sup>0</sup> C.

#### 3.2 Extraction procedure

The Itraconazole solutions were added to blank plasma samples of rabbits, 5 mL of ethyl acetate and 50  $\mu$ L of amyl alcohol were added and adjusted the pH to 7.4 with 0.2 mol NaOH for absolute recovery followed by centrifuge at 2500 r/min at 40 min. The ethyl acetate phase was separated and evaporated under the nitrogen stream, reconstituted with 2 mL of mobile phase and stored at -20<sup>0</sup>C till analyzed by RP-HPLC system.

### 4. Study design and Pharmacokinetic analysis:

#### Methods:

In a single-dose, open-label, crossover study, twelve rabbits bisected randomly in two groups, six rabbits in each group. White Rabbits weighing 1.4-1.6 kg were allowed free access to a normal chow diet and water for at least one week before the experiments. The rabbits were anesthetized with diethyl ether, and the common central ear vein was cannulated with polyethylene tubing for blood sampling. The cannula was flushed and filled with heparinized isotonic saline (100 IU/mL) to prevent the blood from clotting. The rabbits were housed individually in a cage for one day to allow them to recover from surgery.

The rabbits were fasted overnight for at least 6 hour before the experiment, with free access to water. The rabbits were assigned to one of two groups each of 6 rabbits in each group. A minicapsule filled with Spherical agglomerates formulation and marked formulation equivalent to 6.5 mg of Itraconazole was administered orally with a mini capsule injector. Immediately after oral dosing, 5 mL of distilled water was given to each rabbit to help them swallow the minicapsule. About 1 mL of bold was collected from the each rabbit at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24 hour after the oral administration of the drug. About 0.2 mL of heparinized 0.9% NaCl solution was let into the cannula just after each bold sample was taken and centrifuged immediately, the plasma was collected and stored at -20<sup>0</sup> c until HPLC analysis.

The pharmacokinetic parameters of spherical crystals formulation and marketed formulation after the oral administration were determined by non-compartmental analysis using Winnonlin software. The pharmacokinetic parameters estimated in this study are Area under the plasma concentration time curve from 0-24 h ( $AUC_{0-24}$ ), Area under the plasma concentration time curve from 0 hour to infinity ( $AUC_{0-\infty}$ ), elimination half-life( $t_{1/2}$ ), Volume of distribution ( $V_d$ ) and clearance ( $Cl_t$ ), The maximum plasma concentration ( $C_{max}$ )and the time required to reach  $C_{max}$  ( $t_{max}$ ).

WINNONLIN<sup>®</sup> (WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM 1.0 Core Version 04 Jun 2007) is the industry standard used for pharmacokinetic, Pharmacodynamic, and non-compartmental analysis. In addition to its extensive library of built-in PK, PD and PK/PD models.

### 5. Results and Discussions:

#### 5.1 *In vitro* dissolution studies

The results of *in vitro* dissolution studies are shown in Figure 1, Compressibility parameters in table 2. Pure Itraconazole exhibited very less release at the end of 30 min is 66.6%. The drug release at 30

minutes from the spherical crystals prepared in the presence of 1% to 4% w/v PEG 6000 were 65.2, 72.6, 78.6 and 84.4% respectively, which indicates that the spherical crystals prepared with 4% w/v PEG 6000 exhibited maximum drug release i.e. 84.4%.

This indicates that Itraconazole crystals prepared with 4% w/v PEG 6000 exhibit an excellent dissolution and solubility enhancing ability. The mechanism behind the solubility and dissolution rate enhancing effect of Itraconazole in crystals form may resemble the solid dispersion mechanism, although the particle size of the crystals is high. This effect may be due to the improved wettability of the surface of the crystals by the adsorption of PEG 6000 onto the surfaces of crystals. This crystal modification technique can enhance the bioavailability of the drug. Further *in vivo* studies are continued to study the improved solubility and dissolution rate on bioavailability of Itraconazole.

Spherical Crystals	LbD (g/ml)	TbD (g/ml)	Carr's Index	Hausner's ratio (%)	Angle of Repose	True Density (g/ml)	Porosity (%)	Particle Size
PEG 6000 1% W/V	0.42±0.01	0.45±0.01	10.20±1.51	1.18±0.01	29.31±1.23	1.22±0.12	59.55±2.36	201.12±10.18
PEG 6000 2% W/V	0.4±0.02	0.45±0.01	10.10±2.36	1.10±0.02	26.47±2.31	1.25±0.26	65.15±2.16	218.13±12.10
PEG 6000 3% W/V	0.37±0.01	0.41±0.01	10.00±2.12	1.10±0.01	25.61±1.89	1.28±0.1	71.05±3.01	230.25±11.77
PEG 6000 4% W/V	0.39±0.01	0.44±0.01	10.10±2.69	1.00±0.01	22.88±1.65	1.25±0.16	74.32±1.36	235.11±12.23
Pure Itraconazole	0.30±0.01	0.52±0.01	42.00±2.36	1.69±0.03	42.61±1.99	1.55±.46	55.50±2.21	85.55±10.25

Table 2: Micromeritic properties of Itraconazole spherical crystals prepared in the presence of different concentrations of peg-6000 and pure drug

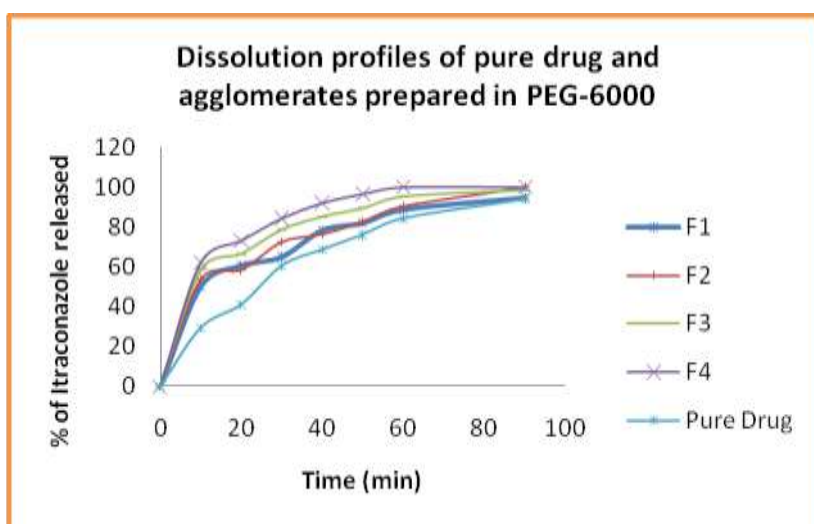


Fig 1 : Dissolution profiles of pure drug and agglomerates prepared in PEG-6000 (1% to 4% w/v).

### 5.3 Comparison of dissolution of compacts and commercial products

Out of all spherical crystals, the agglomerates prepared with 4% w/v PEG 6000 have shown maximum drug release in addition to improving the micromeritic properties of the drug, hence selected as optimized batch. The study the effect of super disintegrants such as Ac-Di-Sol(CCS), Polyplasdone XL (CP) and Explotab (SSG) on the drug release of Itraconazole was done by compressing agglomerates prepared with 4% w/v PEG 6000 and drug with 4% w/w of above said superdisintegrants.

These spherical agglomerated dense compacts (SADC) along with market product were subjected to dissolution studies and maintained same conditions of the spherical agglomerates. The results of

drug release from dense compacts and market product are shown in fig.2. The results displayed that the percent release of Itraconazole from dense compacts and market product, at the end of 30 minutes were found to be 78.8, 93.6, 84.9, 78.6 for dense compacts prepared with 4%w/w Crospovidone, CCS, SSG and commercial product correspondingly. These results indicate that the efficiency of our formulation i.e. dense compacts prepared with 4% CCS exhibited better drug release is compared to other formulations and market product.

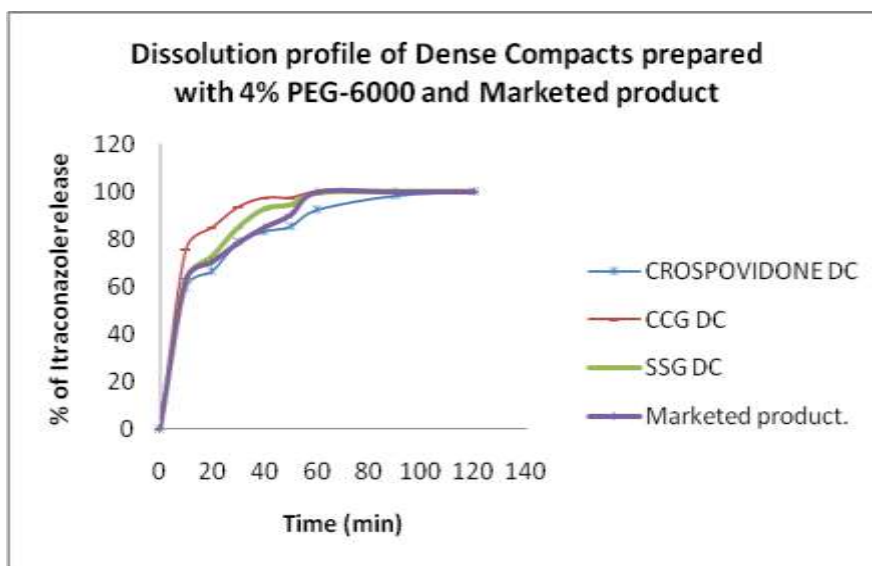


Fig. 2: Dissolution profiles of dense compacts of PEG 6000 spherical crystals and market product.

#### 5.4 Pharmacokinetic analysis:

The concentration of Itraconazole in plasma and the respective average values at different time intervals following the oral administration of selected formulation i.e. Itraconazole spherical agglomerates prepared in 4% PEG 6000 solution compressed with 4% of Ac-Di-Sol and commercial product were evaluated for their pharmacokinetics using Winnonlin software. The average plasma concentration versus time was shown in figures 4,5,6 and log concentration versus time curve show in figure 7. Individual and mean pharmacokinetic parameters ( $AUC_{0-24}$ ,  $AUC_{0-\infty}$ ,  $T_{max}$ ,  $C_{max}$ ,  $K_{el}$ ,  $t_{1/2}$  and  $MRT_{0-24}$ ) calculated from the average serum concentrations from the *in vivo* experiments are in shown in table 3.

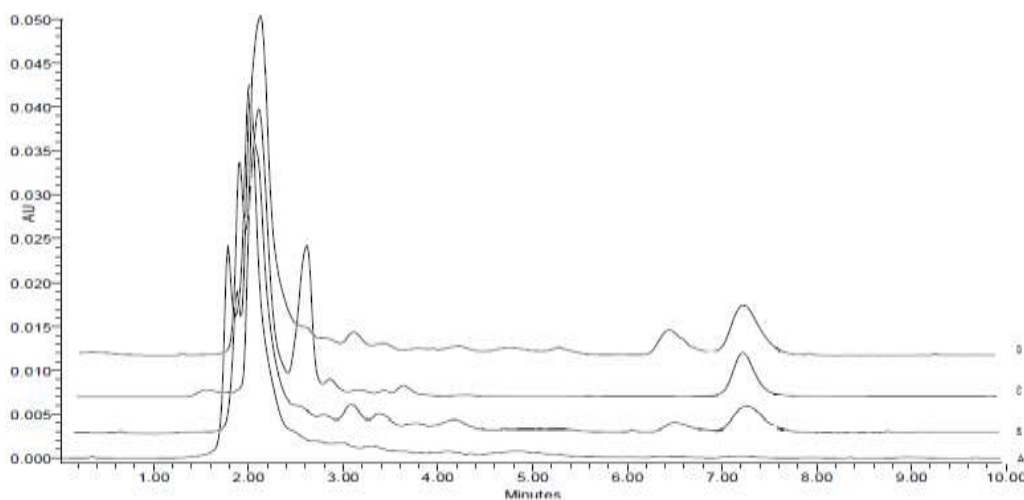


Fig 3: Typical HPLC chromatogram of (a) blank plasma (b) plasma spiked with 10µg /mL of Itraconazole (c) Itraconazole of a rabbit administered with formulation and (d) Itraconazole of a rabbit administered with commercial product.

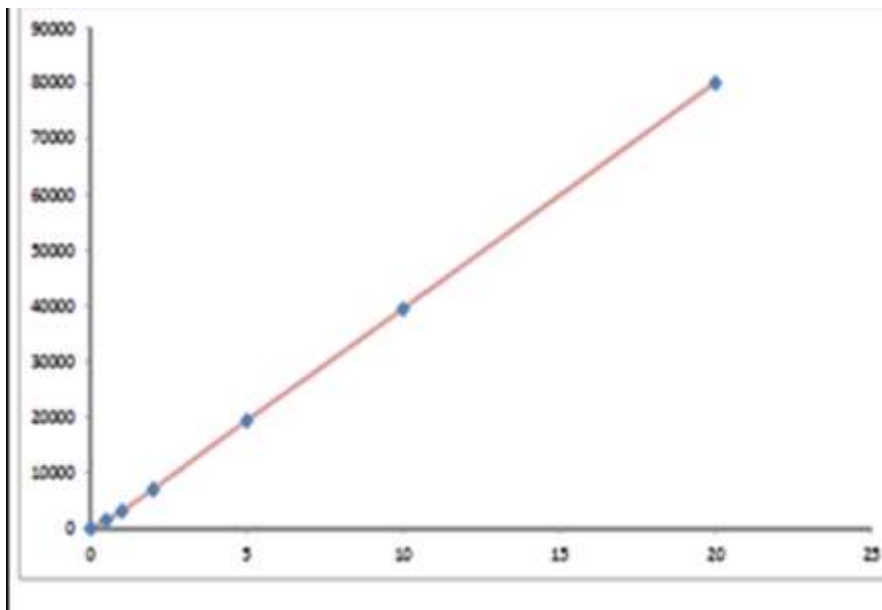


Fig 4: Calibration curve of Itraconazole in rabbit plasma by HPLC method.

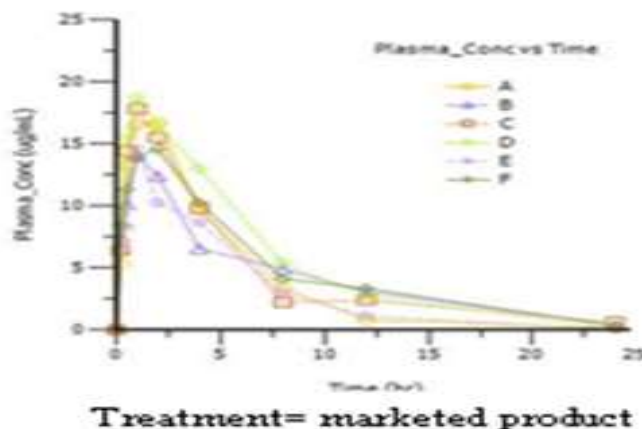


Fig 5: Individual Plasma concentration time curves of Itraconazole followed by oral administration of commercial product

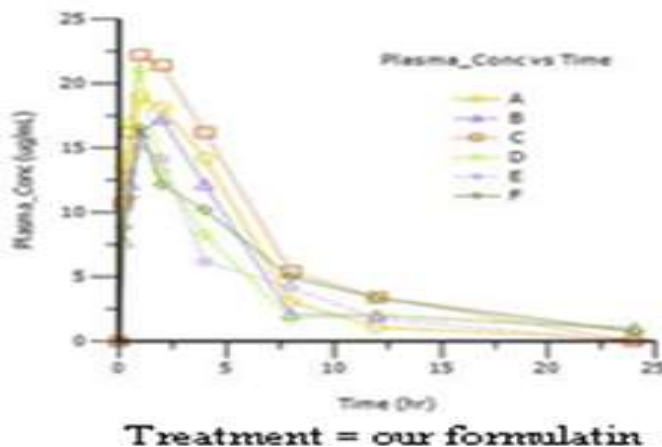


Fig 6: Individual Plasma concentration time curves of Itraconazole followed by oral administration of our formulation.

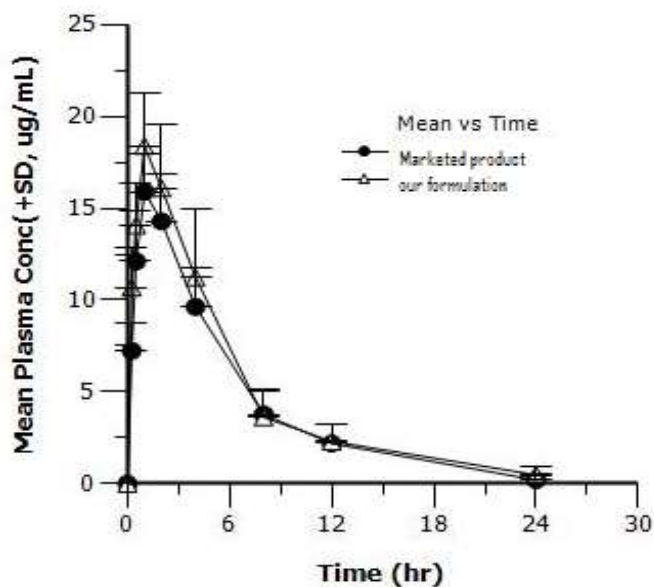


Fig 7: Plasma concentration time curves of Itraconazole followed by oral administration of our formulation.

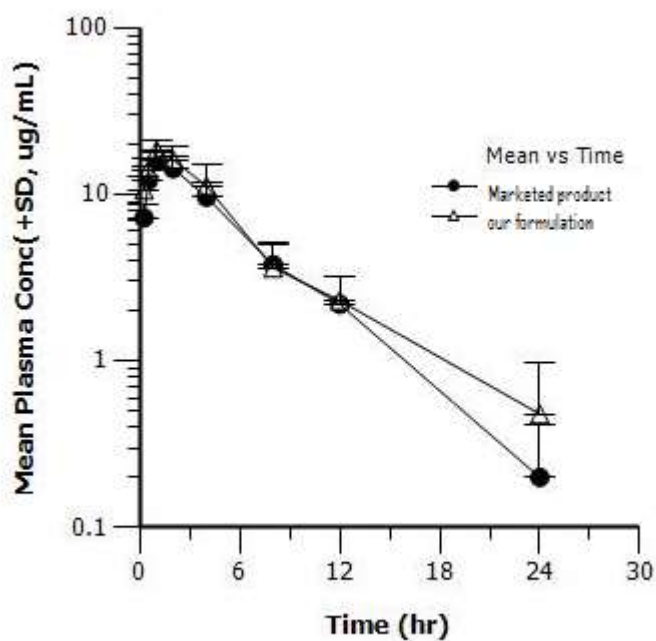


Fig 8: Mean log conc. vs. time curves of Itraconazole followed by oral administration of our formulation.

Table 3: Pharmacokinetic parameters of Itraconazole Commercial product and Spherical Agglomerates (Directly compressible spherical agglomerates).

Pharmacokinetic Parameter	SADC	Commercial product
C <sub>max</sub> (µg/ml)	18.39 ± 2.59	15.09 ± 1.87
T <sub>max</sub> (hrs)	1.16 ± 0.51	1.33 ± 0.50
AUC <sub>0-24</sub> (µg.hr/ml)	115.789 ±3.09	97.334 ±3.18
MRT <sub>0-24</sub> (hrs)	5.253 ±1.02	5.079 ±1.02
K <sub>el</sub> (hrs <sup>-1</sup> )	-0.807 ±0.03	-0.619 ±0.02
t <sub>1/2</sub> (hrs)	0.858 ±0.03	1.11 ±0.03

The C<sub>max</sub> of Itraconazole from selected formulation and commercial product were found to be 18.39 ± 2.59 and 15.09 ± 1.87 µg/mL respectively and the values of the commercial product are below the selected formulations. The t<sub>max</sub> values from selected formulation and commercial product were found to be 1.16 ± 0.51 and 1.33 ± 0.50 hr respectively and the values of the commercial product are below the selected formulations. The K<sub>el</sub> values were found to be -0.807 ±0.03 and -0.619 ±0.02 hr<sup>-1</sup> after oral administration of selected formulation and commercial product respectively calculated from the slope of the terminal portion of log plasma concentration of Itraconazole versus time. The corresponding t<sub>1/2</sub> values were found to be 0.858 ±0.03 and 1.11 ±0.03 hrs respectively. The t<sub>1/2</sub> values are almost similar to the reported values.

The values of AUC<sub>0-24</sub>, and MRT<sub>0-24</sub> of selected formulation and commercial product were 115.789 ±12.9, 97.334 ±7.478 and 5.253 ±1.02, 5.079 ±1.162 respectively.

The AUC values of selected formulation were higher compared to the commercial product indicating the higher bioavailability of the selected formulation. Thus the results of present study indicated the applicability of spherical crystals of Itraconazole as directly compressible material in direct tableting.

## 6. Conclusion:

This study ascertains that the spherical agglomerated dense compacts were successfully formulated. The use of PEG 6000 in the formulation of spherical agglomerates successfully improved bioavailability of the Itraconazole compared to commercial product confirmed by in vivo studies in rabbits. There lies a great promise that spherical agglomeration technology with use of hydrophilic polymers like PEG 6000 will hasten the drug dissolution and bioavailability of miserable water soluble drugs.

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