

## STUDIES ON ORAL BIOAVAILABILITY ENHANCEMENT OF ITRACONAZOLE SALTS BY COMPLEXATION WITH SULFO-BUTYL<sub>7</sub> ETHER B CYCLODEXTRIN

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### ABSTRACT

**Objective:** The main objective of the present study is to improve the solubility and dissolution rate of itraconazole by converting into salts and further preparation of inclusion complexes of salts with Captisol (sulfobutyl ether<sub>7</sub> β-cyclodextrin). The optimized preparation was studied for the improvement oral bioavailability. **Method:** Itraconazole hydrochloride, mesylate and besylate salts were prepared by using addition reaction with hydrochloric acid, methane sulphonic acid and benzene sulphonic acid. Then inclusion complexes of itraconazole salts were prepared with Captisol by using physical mixing, kneading and co-evaporation techniques. The preparations characterized for their physicochemical properties. The optimized preparation was evaluated for *in vivo* bioavailability in rabbit model and compared with pure drug. **Results:** The solubility of prepared salt was found multifold than the solubility of itraconazole. The dissolution studies of itraconazole complexes exhibited high percentage drug dissolution than that of the pure drug which can be attributed to the increase in drug solubility provoked by the complexation technique. Among all complexes, products prepared by kneading method showed higher percentage drug release. The formulations have demonstrated the significant improvement of bioavailability (AUC=6729.1ng/ml) compared to pure drug (AUC=5487.4ng/ml). These results demonstrated the efficacy of complex for the enhancement of oral bioavailability of itraconazole by increasing its aqueous solubility.

**Keywords:** Antifungal, Itraconazole, Hydrochloride Salt, Sulfonate Salt and Captisol.

### 1. INTRODUCTION

Poorly water soluble drugs are posing a problem of satisfactory dissolution within the gastro intestinal tract and there by their oral bioavailability. The recent past has witnessed the modern techniques of drug discovery which lead to an increasing number of drug candidates with unfavorable solubility characteristics<sup>1</sup>. Formulation of such compounds for oral delivery has been the most frequent and greatest challenge to scientists in the pharmaceutical industry. Major problem associated with poorly soluble drugs is lack of dissolution there by results in poor and/or variable bioavailability<sup>2</sup>. Kaplan<sup>3</sup> has suggested that the solubility of a drug more than 10mg/mL at a pH < 7 is expected to have no dissolution

as well as bioavailability related problems but, this could be a problem for drugs whose solubility is below 1mg/mL. Dissolution rate less than 0.1mg/cm<sup>2</sup>/min were likely to give dissolution rate limited absorption. Solubility of a drug is an intrinsic property and it can only be altered by chemical modification of the molecule by salt formation<sup>4-6</sup> or prodrug formation<sup>7</sup>. Dissolution is an extrinsic property which can be modified by various chemical, physical or crystallographic techniques like complexation, particle size reduction, surface or solid state properties. Different techniques have been reported in the literature for improvement of solubility and drug dissolution rates. These techniques are reduction of the particle size by micronisation or nanonisation to

increase the surface area, use of surfactants, Cyclodextrin complexation, pro-drug formation, conversion of crystalline to amorphous forms<sup>8</sup>. Pharmaceutical salts<sup>9</sup> are important in the process of drug development for converting an acidic or basic drug into a salt by a simple neutralization reaction. Using different chemical species to neutralize the parent drug can produce a diverse series of compounds and this process is traditionally being used for modification of the physicochemical, processing, biopharmaceutical or therapeutic properties of drug substances. Each of the individual salts of a particular drug substance can be considered as a unique chemical entity with their own distinctive physicochemical and biopharmaceutical properties<sup>10-12</sup>. It has been estimated that approximately half of all of the active pharmaceutical substances (API) that have been developed were ultimately progressed as pharmaceutically acceptable salts and that salt formation is an integral part of the development process<sup>13,14</sup>. Sulfonic acid salts particularly alkyl sulfonates such as mesylates and besylates generally results in the formation of high melting point API salts with good solubility and stability<sup>15</sup>. Cyclodextrins (CDs) are useful functional excipients that have widespread attention and use. A number of cyclodextrin-based products have reached the market based on their ability to change undesirable physicochemical properties of drugs<sup>16,17</sup>. The formation of inclusion complexes provides numerous advantages in pharmaceutical formulation development  $\beta$ -CD were reported to increase bioavailability of poorly soluble drugs by increasing the drug solubility<sup>18</sup>. The three commonly used cyclodextrins are  $\alpha$ -cyclodextrins comprised of six glucopyranose units,  $\beta$ -cyclodextrins comprised of seven units and  $\gamma$ -cyclodextrins comprised of eight such units<sup>19</sup>. Sulfobutyl ether  $\beta$ -Cyclodextrin (SBE<sub>7</sub>- $\beta$ -CD) [Captisol®]<sup>20-22</sup> is a chemically modified  $\beta$ -cyclodextrins that is a cyclic hydrophilic oligosaccharide which is negatively charged in aqueous media. The solubility in water for Captisol (70 g/100 ml at 25 °C) is significantly higher than  $\beta$ -cyclodextrin (1.85 g/100 ml at 25 °C). It does not exhibit the nephrotoxicity and cytotoxicity which is generally associated with other  $\beta$ -CDs<sup>23-25</sup>. Some of the investigations also reported that the drug inclusion complex with SBE<sub>7</sub>- $\beta$ -CD provided a protective effect against drug-induced cytotoxicity<sup>25</sup>. Based on these advantages, Captisol has been selected to study the effect of improving the physicochemical properties of poorly water-soluble drug itraconazole. Itraconazole (ITR) is a broad-spectrum triazole antifungal agent with poor aqueous solubility<sup>26</sup>.

ITR is weakly basic with pKa of the piperazine ring is 3.7 and highly hydrophobic drug<sup>27</sup>. Because of poor aqueous solubility itraconazole on oral administration results in poor bioavailability and variations in the plasma drug concentrations. ITR has the characteristic of pH dependent solubility having highest solubility at acidic side (4 $\mu$ g/ml) compared to basic pH (1 $\mu$ g/ml). However, because of highly lipophilic nature (log P= 6.2) it can easily penetrate into intestinal membrane. This indicates the poor aqueous solubility is the main reason for lower plasma concentrations. Various techniques<sup>8</sup> have been reported for enhancing the solubility and bioavailability of itraconazole, but the salt formation<sup>13,14</sup> and inclusion complexes<sup>18</sup> showed some promising results. Keeping these in view the present work planned with an objective to synthesize Itraconazole mesylate and Itraconazole besylate salt forms from Itraconazole. Further salt forms have studied for improvement of solubility and dissolution by preparing inclusion complexes with Sulfobutyl<sub>7</sub> Ether  $\beta$ -Cyclodextrin (Captisol®) using physical mixing, kneading and co-evaporation techniques. These preparations are characterized by X-ray diffraction, Fourier Transformed Infrared spectroscopy and also evaluated for solubility, drug content, dissolution studies and *in vivo* pharmacokinetics.

## 2. MATERIALS AND METHODS

Itraconazole was a gift sample obtained from Pharmatech, Hyderabad, and Sulfobutyl<sub>7</sub> Ether  $\beta$ -Cyclodextrin (Captisol®) (average molecular weight 2,163 and degree of substitution 6.5) was obtained from Cydex laboratories. Benzene sulfonic acid (A.R. grade) and Methane sulfonic acid (A.R. grade) were purchased from Merck. All other chemicals used in this study were of analytical grade.

### HPLC analysis of Plasma Samples

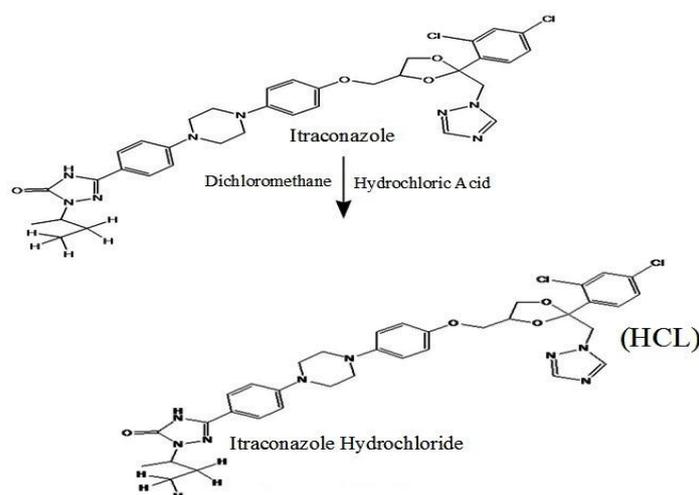
Itraconazole in rabbit plasma samples were estimated by using HPLC method. Waters HPLC system with PDA detector and Discovery C18 column (250 mm  $\times$  4.6 mm ID, 4.6  $\mu$ m) was used in the present study. The solvent system used was 0.1% v/v perchloric acid and acetonitrile as mobile phase in ratio 40:60 at flow rate 1ml per minute. A wave length 287 nm was used for detection. Blood collected in pre coated 2.0 ml heparin tubes from marginal ear vein of rabbit. The study was approved by the Institutional Animal Ethics Committee. The blood centrifuged in cooling centrifuge at 4000 rpm and yellow supernatant plasma was collected. Standard solutions of itraconazole were prepared with water and acetonitrile in concentrations range from 0.1 $\mu$ g/ml to 2 $\mu$ g/ml.

Voriconazole was used as internal standard and spiked of same standardized concentrations of Itraconazole.

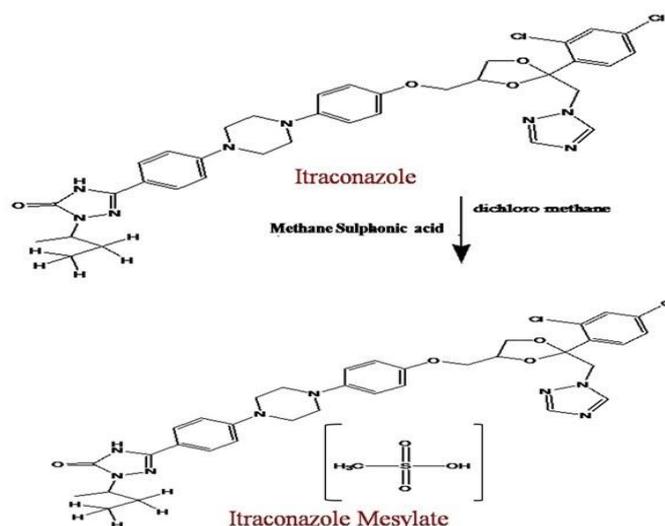
### Preparation of Itraconazole Salts

Itraconazole hydrochloride (ITRH), Itraconazole mesylate (ITRM) and Itraconazole besylate (ITRB) salts were synthesized from itraconazole (ITR) by acid addition reaction using hydrochloric acid, methane sulfonic acid and benzene sulfonic acid (Figure.1, 2 & 3). Itraconazole salts were synthesized from a modified method by using acid addition reaction method<sup>28-30</sup>. Itraconazole pure drug was accurately weighed about 1g (1.4 mmol) and suspended in about 10 ml of dichloromethane. In case of ITRM preparation, accurately weighed about 1 gm of ITR (1.4 mmol) and was dissolved in about 10 ml of dichloromethane in a rotary evaporator flask. To this solution about 400 mg of concentrated

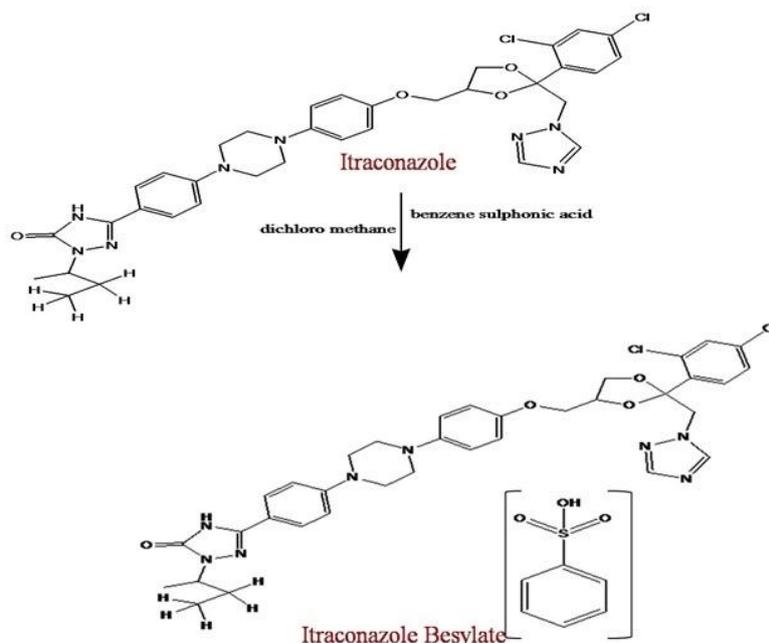
hydrochloric acid (11.42 mmol) was added and dissolved. The above suspension was heated at 50 °C for 1hr under reflux using rotary evaporator. After one hour 700 mpa vacuum was applied while reaction. The reaction was continued for one hour to form a precipitate of salt. The mixture was allowed to stand overnight at room temperature. The precipitated product was collected, dried at 60°C for 1 hour and shifted through #100 mesh sieve. ITRM and ITRB salts were prepared by following the similar procedure as mentioned above for ITRH salt by taking 1 gm of ITR (1.4 mmol) suspended in about 10 ml of dichloromethane and to this solutions about 400 mg of methane sulfonic acid (4.16 mmol) and 600 mg of benzene sulfonic acid (3.9 mmol) were added and dissolved. The final products were stored in an air tight container and then placed in desiccators.



**Fig. 1: Synthesis of Itraconazole Hydrochloride from Itraconazole**



**Fig. 2: Synthesis of Itraconazole Mesylate from Itraconazole**



**Fig. 3: Synthesis of Itraconazole Besylate from Itraconazole**

### Solubility Studies

Solubility studies for pure ITR, ITRH, ITRM and ITRB were carried in purified water and simulated gastric fluid (pH 1.2 - 0.1 N Hydrochloric Acid). In each case excess amount of sample was added to 10 ml of solvent and agitated at 37°C in a rotary test tube shaker for 24 hrs. After equilibration, the samples were filtered using 0.45 µm Millipore filters, suitable diluted and analyzed for the content itraconazole by measuring the absorbance at 258 nm using Shimadzu UV-Visible spectrophotometer<sup>31</sup>.

### Phase Solubility Studies

A phase solubility study<sup>32-36</sup> was carried out to investigate the effect of Captisol on the solubility of ITR, ITRH, ITRM and ITRB using the method reported by Higuchi and Connors. Captisol was added and dissolved in simulated gastric fluid (pH 1.2-0.1N HCL) to obtain concentrations of 5, 10, 20, 40 and 80 mM. To each of these solutions excess amounts of ITR, ITRH, ITRM and ITRB were added separately and shaken using orbit shaker at 25°C for 72 hr. After equilibrium, the solutions were filtered using 0.45µ filters and diluted suitably to determine the concentration of ITR, ITRH, ITRM and ITRB at 258 nm using UV-Visible spectrophotometer. The graphs were plotted between ITR, ITRH, ITRM and ITRB concentrations (in mM) against the concentration of Captisol (in mM). The stability constant for the complex was determined from the graph using the following equation.

$$K_s = \frac{\text{Slope}}{S_0(1 - \text{Slope})}$$

The slope was obtained from the graph and  $S_0$  was the equilibrium solubility of ITR, ITRH, ITRM and ITRB in 0.1 N HCl.

### Preparation of Inclusion Complexes

The inclusion complexes of ITR, ITRH, ITRM and ITRB with Captisol (1:2 and 1:3 ratios) were prepared by using physical mixing, kneading and co-evaporation technique<sup>37</sup>. Physical mixture was prepared by simple mixing in a mortar with pestle for 10 min. The powders of ITR, ITRH, ITRM, ITRB and Captisol of required molar ratios are simply mixed in mortar with pestle and then sieved through 100 #. Kneaded (KN) product was obtained by triturating equimolar quantities of ITR, ITRH, ITRM, ITRB and Captisol of required molar ratios in a mortar with a small volume of solvent blend of water: methanol: dichloromethane at a volume ratio of 2:5:3. During this kneading process few drops of solvent were introduced to maintain a suitable consistency. The resulting mass was dried in an oven at 55 °C until they get dry and the solid was finally grounded and then sifted through #100 sieve. In co evaporation technique, aqueous solution of Captisol was added to the solution of ITR, ITRH, ITRM and ITRB in a solvent blend of methanol: dichloromethane at a volume ratio of 2:3. The resultant mixture was stirred for 1 hr and evaporated at a temperature

of 55 °C until dry. The dried mass was pulverized and sifted through #100 sieve.

#### Fourier Transform Infra Red Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) spectra of ITR, ITRH, ITRM, ITRB, Captisol, ITR-Captisol complexes, ITRH-Captisol complexes, ITRM-Captisol complexes and ITRB-Captisol complexes were recorded using a Fourier Transform Infrared spectrophotometer (Perkin Elmer, Spectrum Two). The samples were scanned from 400–4000  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$  by using KBr pellet technique.

#### X-ray diffraction analysis

Powder X-ray diffraction (XRD) patterns of ITR, ITRH, ITRM, ITRB, Captisol, ITR-Captisol complexes, ITRH-Captisol, ITRM-Captisol complexes and ITRB-Captisol complexes were recorded on a PAN Analytical X'Pert powder X-ray diffractometer (X'Pert PRO) using Ni-filtered, Cu K $\alpha$  radiation, a voltage of 40 kV and 60 mA current. The scanning rate was 4°/min over the diffraction angle range ( $2\theta$ ) of 3–50°.

#### Drug Content Estimation

Accurately weighed 50 mg of the sample and transferred into a 50 ml volumetric flask. Then 25 ml of 50% methanol:0.1N HCl mixture was added and shaken for 15 minutes to completely dissolve the drug. The volume is made up to 50 ml with 50% methanol:0.1N HCl mixture. The resulted solution was filtered through 0.45  $\mu\text{m}$  filter and suitable diluted and analyzed for the content itraconazole by measuring the absorbance at 258 nm using Shimadzu UV-Visible spectrophotometer. The drug content of all the inclusion complexes was estimated by following the same method.

#### In vitro Dissolution Studies

*In vitro* dissolution studies<sup>38</sup> were carried out in 900 ml of simulated gastric fluid of pH 1.2 using USP Type-II (Paddle) dissolution test apparatus (M/s. Electro Lab India). Sample equivalent to 100 mg of ITR, a speed of 75 rpm and a temperature of 37 $\pm$ 0.5 °C were used in each test. A 5 ml aliquot was withdrawn at different time intervals, filtered and replaced with 5 ml of fresh dissolution medium. The filtered samples were suitably diluted whenever necessary and assayed for ITR by measuring absorbance at 258 nm. The dissolution studies were carried for the pure ITR and the prepared ITR salts inclusion complexes. Commercial ITR capsules Sporonax<sup>®</sup> was also evaluated for dissolution for comparison. All the dissolution experiments were conducted in triplicate and

the mean values are reported.

#### In Vivo Pharmacokinetic Studies

In this study New Zealand white rabbits were selected and separated into two groups. The itraconazole and itraconazole hydrochloride complex (kneading 1:2 ratio) were administered in the oral cavity for Group-I and Group-II animals respectively. The blood samples of 1.5 ml collected at different time intervals and centrifuged for separation of plasma. Itraconazole contents of the plasma samples were determined by HPLC method. Pharmacokinetic parameters were calculated for two groups.

### 3. RESULTS AND DISCUSSION

#### Solubility Studies

The solubility of ITR was found to be 1.388  $\mu\text{g}/\text{mL}$  in purified water and 7.59  $\mu\text{g}/\text{mL}$  in 0.1N HCl. After conversion into salt forms the solubility of ITRH in water was increased from 1.388  $\mu\text{g}/\text{mL}$  to 23.86  $\mu\text{g}/\text{mL}$  at 37° C and in simulated gastric fluid the solubility was increased from 7.59  $\mu\text{g}/\text{mL}$  to 93.60  $\mu\text{g}/\text{mL}$ . In similarly the solubility of ITRM was found to be 165.86  $\mu\text{g}/\text{mL}$  in purified water and 402.6  $\mu\text{g}/\text{mL}$  in 0.1 N HCl respectively. The solubility of ITRB in purified water was found to be 191.64  $\mu\text{g}/\text{mL}$  and 508.7  $\mu\text{g}/\text{mL}$  in 0.1N HCl. These results clearly indicated that prepared salts have considerable influence on improvement of ITR solubility.

#### Phase Solubility Studies

The effect of Captisol on the aqueous solubility of ITR, ITRH, ITRM and ITRB were evaluated using the phase solubility method. The results (Table 1) showed an increase in the solubility of ITR, ITRH, ITRM and ITRB with increase in Captisol concentration which indicates the effect of complexation. According to Higuchi and Connors, phase solubility study indicated that the curves can be classified as the AP type (the solubilizer was proportionally more effective at higher concentrations, shows in phase-solubility diagrams (in Figure 5). The positive curvature indicated that the existence of soluble complexes is with an order greater than one. Therefore, the theoretical molar ratio (1:2 and 1:3) was chosen to prepare the solid complexes through different methods. The slope value were lower than one i.e., for ITR, ITRH, ITRM and ITRB was 0.7801, 0.035, 0.0386 and 0.0106 respectively. The apparent stability constant (KS) of ITR: Captisol, ITRH:Captisol, ITRM:Captisol and ITRB: Captisol complex were obtained as 51.322  $\text{M}^{-1}$ , 254.73  $\text{M}^{-1}$ , 213  $\text{M}^{-1}$  and 375  $\text{M}^{-1}$  from the initial linear plot of the phase-solubility diagrams.

Table 1: Phase Solubility Studies

S. No	Concentration Captisol (mM)	Concentration ITR (mM)	Concentration ITRH (mM)	Concentration ITRM (mM)	Concentration ITRB (mM)
1	0	$6.912 \times 10^{-5}$	0.00019	0.193	0.19
2	5	0.00312	0.359	0.386	0.616
3	10	0.00493	0.550	0.503	1.160
4	20	0.00884	1.587	0.734	2.537
5	40	0.0222	2.589	1.658	4.253
6	80	0.0552	34.087	3.080	7.810
Stability Constant ( $k_s$ )		$51.322 \text{ M}^{-1}$	$254.73 \text{ M}^{-1}$	$51.322 \text{ M}^{-1}$	$375 \text{ M}^{-1}$

### FTIR Spectroscopy

Infra red spectra of pure drug (shown in Figure 4) indicated the presence of characteristic peaks of carboxylate group (O-C-O) in the range of 1550-1660 $\text{cm}^{-1}$ , C-N stretch from 1073 $\text{cm}^{-1}$ , chlorine group at 700-850 $\text{cm}^{-1}$ , benzene moiety from 3100-300  $\text{cm}^{-1}$ . The salt forms itraconazole mesylate and itraconazole besylate have got a characteristic peak of S=O group in the range of 1345- 1365. FTIR studies

revealed that Itraconazole HCL showed two typical bands at 3369 and 3283  $\text{cm}^{-1}$  due to N-H primary stretching vibration and a band at 3170  $\text{cm}^{-1}$  due to N-H secondary stretching and characteristics bands at 1623 and 1560  $\text{cm}^{-1}$  assigned to C=N stretching. FTIR results suggested that there is no significant chemical interaction between the drug and the Captisol complexed products, which confirms the stability of drug in the powdered form.

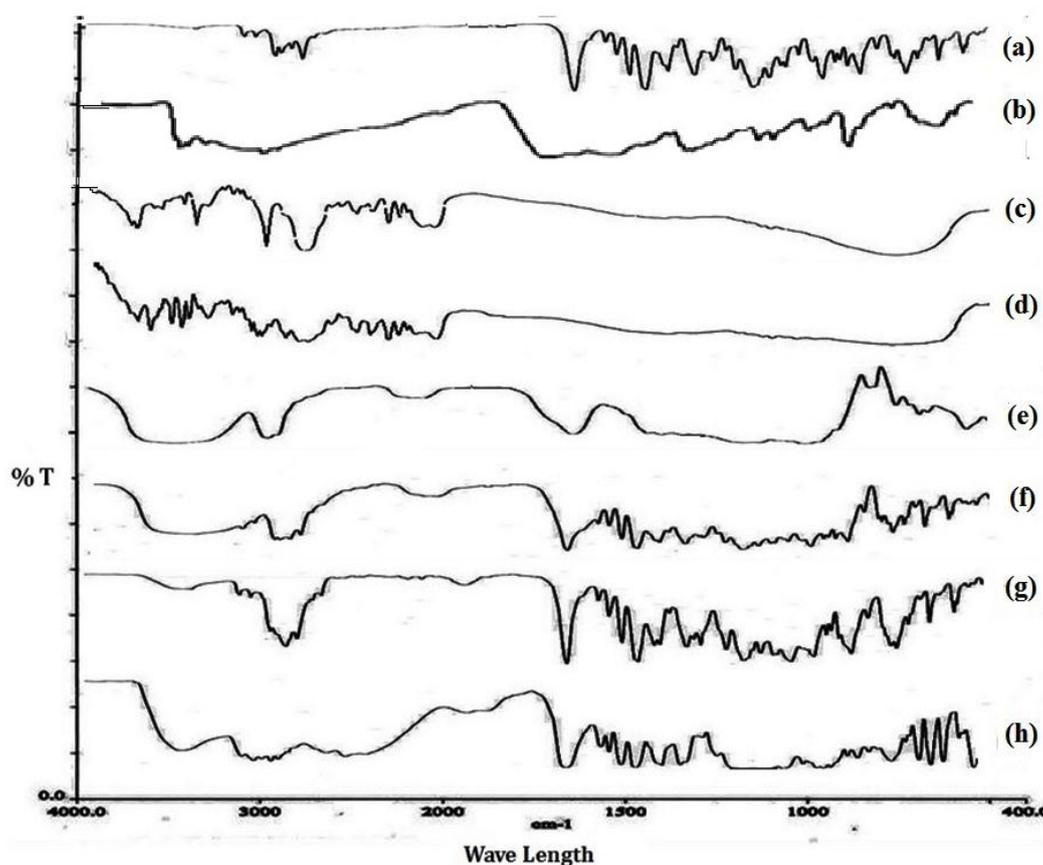


Fig. 4: IR spectra of (a) ITR (Pure Drug) (b) ITRH (c) ITRM (d) ITRB (e) Captisol (SBE7  $\beta$ -CD) (f) ITRH-C-KN (g) ITRM-C-KN (h) ITRB-C-KN

### X-ray powder diffraction (XRD)

The solid-state form, like as crystalline, polymorphs, solvates or amorphous solids of a drug substance, can have a significant impact on drug's solubility, dissolution rate, stability in a pharmaceutical formulation and bioavailability. A crystal has an ordered arrangement of molecules and atoms, maintained in contact through non-covalent interactions. On the other hand, amorphous solids are characterized by a random state. Although the amorphous solids are often susceptible to changes during storage, the amorphous form of a drug is generally more

soluble, due to free energies involved in the dissolution process. This characteristic of solubility is a useful property, particularly if the drug has low aqueous solubility. The XRD pattern of ITR and ITR complexes samples are shown in Figure 5. The pure drug spectra has shown intense and sharp at 16, 20 and 28° 2θ indicating its crystalline nature. The XRD patterns of salts and the complexed products have been found to have no peaks indicating their amorphous nature and inclusion complex formation with Captisol.

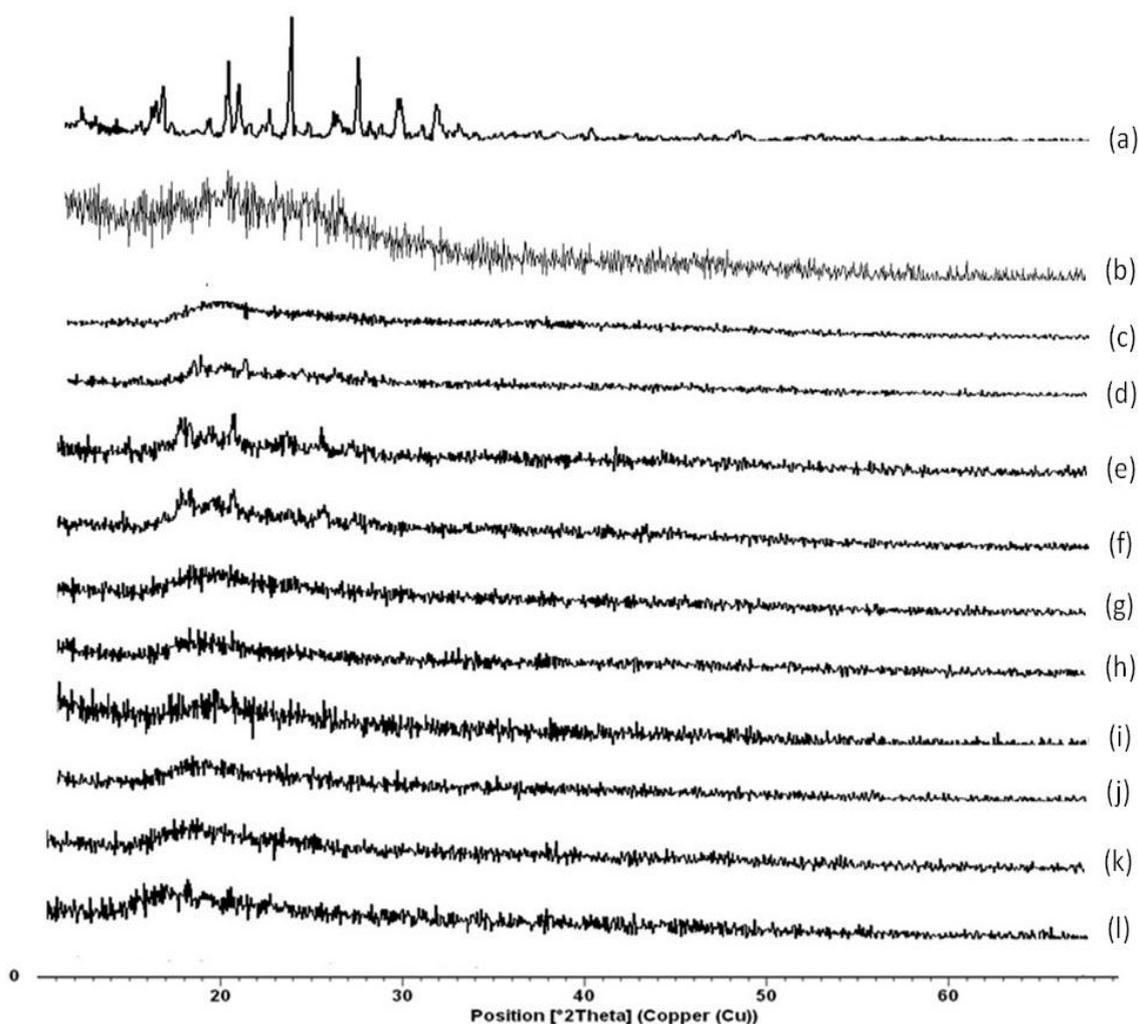


Fig. 5: XRD Pattern of (a) ITR (b) ITRM (c) ITRB (d) ITRH (e) ITRH-C-PM (f) ITRH-C-EV (g) ITRH-C-KN (h) ITRM-C-PM (i) ITRM-C-EV (j) ITRM-C-KN (k) ITRB-C-EV (l) ITRB-C-KN

### Drug Content Estimation

The percentage drug content of different itraconazole complexes are shown in Table 2. The drug content was found to be in the range

of  $75.66 \pm 0.34\%$  w/w to  $99.45 \pm 0.18\%$  w/w. The low standard deviation values indicated the uniformity of drug content of the prepared complexes.

**Table 2: Different Itraconazole Complexes and their Drug Content**

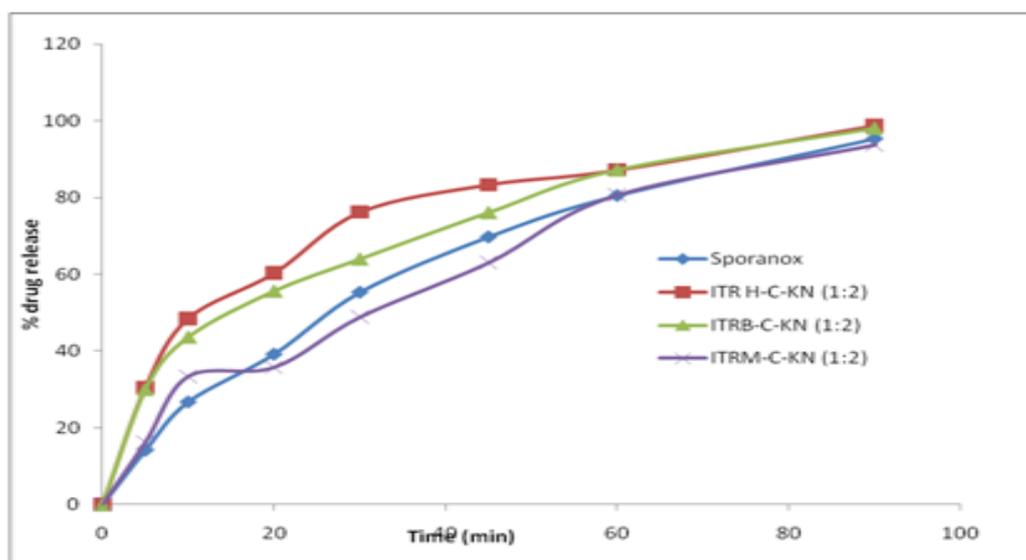
Complexes	Method	Terminology	Drug content (% w/w)	
			1:2	1:3
ITR + C	KN	ITR-C-KN	97.23±0.12	87.23±0.17
	EV	ITR-C-EV	93.83±0.14	81.36±0.23
	PM	ITR-C-PM	85.89±0.21	75.66±0.34
ITRH + C	KN	ITRH-C-KN	98.45±0.58	93.86±0.52
	EV	ITRH-C-EV	94.88±0.21	91.39±0.24
	PM	ITRH-C-PM	89.04±0.41	90.11±0.45
ITRM + C	KN	ITRM-C-KN	98.64±0.19	95.64±0.14
	EV	ITRM-C-EV	94.55±0.15	94.55±0.16
	PM	ITRM-C-PM	90.99±0.12	77.94±0.33
ITRB + C	KN	ITRB-C-KN	99.45±0.18	93.86±0.25
	EV	ITRB-C-EV	94.88±0.20	91.39±0.26
	PM	ITRB-C-PM	89.04±0.29	80.11±0.21

### **In Vitro Dissolution Study of Complexes**

The dissolution profiles of itraconazole from pure drug and different complexes prepared by physical mixture, kneading technique, co-evaporation techniques. Itraconazole pure drug has dissolved only 16.89 % in 90 minutes indicating the poor solubility and thereby dissolution. The dissolution of 1:2 and 1:3 weight ratio of ITR:captisol physical mixture complexes in 90 minutes is 18.86 % and 21.31 % w/w respectively, kneading method complexes showed 32.84 % and 49.87 % w/w drug release and the co-evaporation complexes showed dissolution of 26.61 % and 42.34 % w/w respectively. The data indicated that the pure drug complexes with captisol could not able to increase the dissolution to the required level.

The dissolution of 1:2 and 1:3 weight ratio ITRH : captisol physical mixture complexes in 90 minutes is 50.12 % and 44.19 % w/w respectively, kneading method complexes showed 98.72 % and 89.72 % w/w drug release and the co-evaporation complexes showed

dissolution of 73.96 % and 70.68 % w/w respectively. The dissolution of 1:2 and 1:3 weight ratio ITRM : captisol physical mixture complexes in 90 minutes is 51.58 % and 38.90 % w/w respectively, kneading method complexes showed 93.81 % and 79.65 % w/w drug release and the co-evaporation complexes showed dissolution of 71.96 % and 88.43 % w/w respectively. The dissolution of 1:2 and 1:3 weight ratio ITRB : captisol physical mixture complexes in 90 minutes is 57.36 % and 48.83 % w/w respectively, kneading method complexes showed 98.14 % and 90.73 % w/w drug release and the co-evaporation complexes showed dissolution of 67.92 % and 85.64 % w/w respectively. The data clearly indicated that the itraconazole hydrochloride, mesylate and besylate salt complexes with Captisol can significantly increase the dissolution to the required level. For comparison the dissolution of commercial Sporanox capsules also performed which showed 95.38 % ITR release in 90 minutes (Shown in Figure 6).

**Fig. 6: Percentage drug release of complexes comparison with Sporanox®**

### Kinetics, Dissolution efficiency ( $DE_{90}$ ), Similarity Factor and Difference Factor

The kinetics of ITR release from complexes was studied by subjecting the dissolution data to zero order, first order kinetics (as shown in Table 3). The results indicated that the drug release follows first order kinetics. The mechanism of drug release was found to be by diffusion. The correlation values peppas equation indicated the dissolution follows fick's law of diffusion. The study clearly indicated the usefulness of itraconazole hydrochloride, mesylate and besylate salt complexes with sulpho butyl<sub>7</sub> ether  $\beta$  CD in improving the solubility and dissolution rate of itraconazole.

The dissolution efficiency ( $DE_{90}$ )<sup>39</sup> at 90 minutes was calculated and the values are shown in Table 4 and Figure 7. Pure itraconazole showed  $DE_{90}$  13.84%, ITRH:captisol (1:2 weight ratio) complexes prepared by kneading method showed 74.80%, ITRM:captisol (1:2 weight ratio) complexes prepared by kneading showed 67.42% and ITRB:captisol (1:2 weight ratio) complexes

prepared by kneading showed 73.97%.  $DE_{90}$  of Sporonax capsules in 69.48% and the data indicates that the itraconazole hydrochloride, mesylate and besylate salt complexes with captisol prepared by kneading technique are efficient in improving the dissolution of ITR and are comparable with commercial Sporonax capsules.

The difference factor (f1) and similarity factor (f2)<sup>40</sup> values of ITRH: Captisol (1:2 molar ratio) prepared by kneading method showed 09 and 62 (Shown in Table 4). In similar manner ITRB: Captisol (1:2 molar ratio) prepared by kneading method showed 9 and 65; difference factor (f1) and similarity factor (f2) values of ITRM: Captisol (1:2 molar ratio) prepared by kneading method showed 9 and 64. These values have indicated the equivalence of dissolution profile for ITRH:Captisol (1:2 molar ratio), ITRB:Captisol (1:2 molar ratio) and ITRM:Captisol (1:2 molar ratio) prepared by kneading method to that of commercial capsules Sporanox®.

**Table 3: Dissolution Kinetics of Itraconazole**

Sample	Zero order	First order	Peppas	Higuchi	First order rate constant ( $k_1$ )	n- value
ITR	0.7398	0.7633	0.9731	0.9452	0.0017	0.3393
Sporonax	0.9492	0.99622	0.9899	0.9721	0.0293	0.6897
ITR-C-PM (1:2)	0.9693	0.9680	0.9767	0.9163	0.0021	0.6514
ITR-C-PM (1:3)	0.8105	0.8309	0.9391	0.9626	0.0023	0.5553
ITRH-C-PM (1:2)	0.8340	0.8890	0.9830	0.9760	0.0060	0.485
ITRH-C-PM (1:3)	0.7490	0.8000	0.9460	0.9410	0.0040	0.4550
ITRM-C-PM (1:2)	0.7185	0.7873	0.9267	0.9317	0.0069	0.4255
ITRM-C-PM (1:3)	0.7487	0.8078	0.9857	0.9547	0.0046	0.3487
ITRB-C-PM (1:2)	0.8215	0.9070	0.9925	0.9827	0.0069	0.4017
ITRB-C-PM (1:3)	0.8233	0.8958	0.9870	0.9760	0.0046	0.3565
ITR-C-KN (1:2)	0.7296	0.7720	0.9477	0.9306	0.0023	0.3649
ITR-C-KN (1:3)	0.7688	0.8469	0.9857	0.9620	0.0069	0.3712
ITRH-C-KN (1:2)	0.8930	0.9180	0.9950	0.9970	2.3210	0.393
ITRH-C-KN (1:2)	0.7660	0.9650	0.9520	0.9540	1.9340	0.3970
ITRM-C-KN (1:2)	0.9311	0.9691	0.9646	0.9762	0.0161	0.5779
ITRM-C-KN (1:3)	0.8361	0.9654	0.9543	0.9847	0.0161	0.4974
ITRB-C-KN (1:2)	0.9021	0.9583	0.9922	0.9892	0.0418	0.5546
ITRB-C-KN (1:3)	0.8592	0.9891	0.9767	0.9890	0.0253	0.5064
ITR-C-EV (1:2)	0.9160	0.9373	0.9875	0.9848	0.0023	0.6526
ITR-C-EV (1:3)	0.8420	0.8908	0.9694	0.9804	0.0046	0.5416
ITRH-C-EV (1:2)	0.810	0.937	0.973	0.974	1.658	0.4430
ITRH-C-EV (1:3)	0.743	0.873	0.946	0.943	1.547	0.4090
ITRM-C-EV (1:2)	0.9229	0.9866	0.9928	0.9866	0.0138	0.6005
ITRM-C-EV (1:3)	0.8660	0.9896	0.9845	0.9855	0.0230	0.4808
ITRB-C-EV (1:2)	0.8968	0.9693	0.9826	0.9857	0.0115	0.5515
ITRB-C-EV (1:3)	0.9195	0.9953	0.9945	0.9905	0.0207	0.5490

Table 4: Dissolution Parameters of Itraconazole

Sample	Dissolution Efficiency (DE <sub>90</sub> %)	Difference Factor (f <sub>1</sub> )	Similarity Factor (f <sub>2</sub> )
ITR	13.84	78	17
Sporonax	69.48	-	-
ITR-C-PM (1:2)	14.87	85	16
ITR-C-PM (1:3)	17.19	74	18
ITRH-C-PM (1:2)	40.38	38	31
ITRH-C-PM (1:3)	37.69	41	29
ITRM-C-PM (1:2)	43.61	33	33
ITRM-C-PM (1:3)	32.48	49	25
ITRB-C-PM (1:2)	45.83	32	35
ITRB-C-PM (1:3)	38.85	42	29
ITR-C-KN (1:2)	25.75	56	23
ITR-C-KN (1:3)	38.04	38	31
ITRH-C-KN (1:2)	74.80	09	62
ITRH-C-KN (1:3)	72.86	17	48
ITRM-C-KN (1:2)	67.42	9	64
ITRM-C-KN (1:3)	61.86	17	51
ITRB-C-KN (1:2)	73.97	9	65
ITRB-C-KN (1:3)	70.16	10	60
ITR-C-EV (1:2)	19.55	72	19
ITR-C-EV (1:3)	33.32	50	26
ITRH-C-EV (1:2)	58.88	18	48
ITRH-C-EV (1:3)	58.75	20	46
ITRM-C-EV (1:2)	52.97	23	42
ITRM-C-EV (1:3)	68.68	9	62
ITRB-C-EV (1:2)	51.19	26	40
ITRB-C-EV (1:3)	63.22	11	59

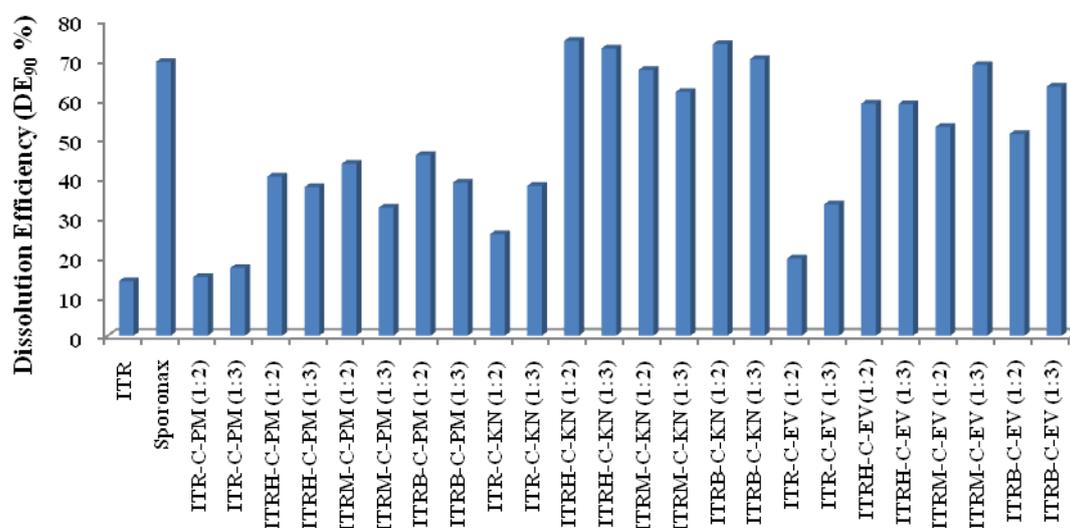


Fig. 7: Comparison of ITR and their Salts Complexes with Dissolution Efficiency

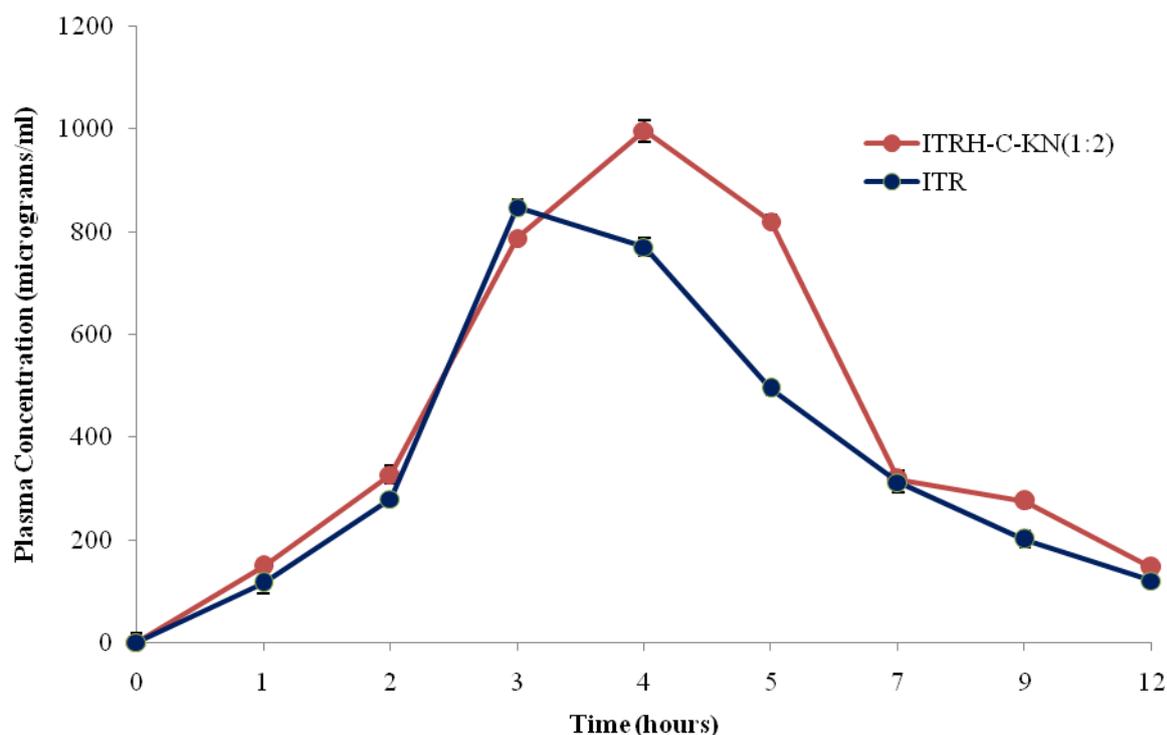
### In Vivo Pharmacokinetic Evaluation

The plasma concentration time profiles of itraconazole in rabbit for itraconazole hydrochloride complex (Kneading 1:2 ratio) and itraconazole is shown in Table 4, Figure 8. The pharmacokinetic parameters were calculated and the data is shown in Table 5. The *in vivo* data showed itraconazole hydrochloride complex (Kneading 1:2 ratio) has highest peak plasma concentration in 4.0 h

when compared with itraconazole pure drug. The results also showed AUC, AUMC and MRT were more for itraconazole hydrochloride complex (Kneading 1:2 ratio) compared to itraconazole pure drug. Statistical data analysis (shown in Table 5) indicated that there was a significant difference in  $K_a$ ,  $K_{el}$ ,  $MRT_{0-24}$  and  $MRT_{0-\infty}$  values among itraconazole hydrochloride complex (Kneading 1:2 ratio) and itraconazole.

**Table 4: Plasma Concentration Time Profiles of Itraconazole**

Time (h)	Plasma Concentration (ng/ml)	
	ITRH-C-KN(1:2)	ITR
1	148 ± 15.6	116 ± 18.3
2	327 ± 11.3	279 ± 21.5
3	787 ± 18.5	847 ± 12.3
4	996 ± 9.4	770 ± 14.6
5	820 ± 21.3	495 ± 17.3
7	318 ± 12.4	312 ± 14.2
9	276 ± 16.7	201 ± 18.6
12	146 ± 14.5	120 ± 15.8

**Fig. 8: In Vivo Pharmacokinetic Parameters (Mean ± SD) for Itraconazole****Table 5: Statistical Analysis of Pharmacokinetic Parameters of ITR and ITR-C-KN(1:2)**

Pharmacokinetic Parameter	ITRH-C-KN(1:2)	ITR	ITRH-C-KN(1:2) Vs. ITR
$C_{max}$ (ng/ml)	996 ± 9.2	847 ± 20	Significant (p=0.020)
$t_{max}$ (h)	4.00 ± 0.00	3.00 ± 0.00	-
$K_a$ (1/h)	0.887 ± 0.006	1.273 ± 0.130	Not Significant (p=0.104)
$K_{el}$ (1/h)	0.161 ± 0.010	0.189 ± 0.010	Not Significant (p=0.197)
$t_{1/2\text{ elim}}$ (h)	4.299 ± 0.187	3.670 ± 0.200	Significant (p=0.009)
$AUC_{0-24}$ (ng h/ml)	5821.5 ± 100	4852 ± 40	Significant (p=0.045)
$AUC_{0-\infty}$ (ng h/ml)	6729.1 ± 19.07	5487.4 ± 100	Significant (p=0.020)
$AUMC_{0-24}$ (ng h <sup>2</sup> /ml)	29259.33 ± 34.5	34507.6 ± 300	Significant (p=0.014)
$AUMC_{0-\infty}$ (ng h <sup>2</sup> /ml)	45744.65 ± 152.5	34507.6 ± 300	Significant (p=0.012)
$MRT_{0-24}$ (h)	5.026 ± 0.079	4.847 ± 0.150	Not Significant (p=0.120)
$MRT_{0-\infty}$ (h)	6.800 ± 0.021	6.288 ± 0.200	Not Significant (p=0.129)

#### 4. CONCLUSION

Based on present study and results it is concluded that formation of itraconazole hydrochloride, besylate and mesylate salt forms could significantly improve the solubility and dissolution rate of itraconazole. These results also suggested that an itraconazole hydrochloride salt complex of sulfo derivative cyclodextrin (Captisol) prepared by kneading technique has greater solubility and dissolution rate when compared with pure drug Itraconazole. The *in vivo* pharmacokinetic data

also clearly indicated the improvement of oral bioavailability of ITRH-C-KN complexes.

#### 6. CONFLICT OF INTEREST

There is no conflict of Interest.

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