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Preparation and Evaluation of Itraconazole Cyclodextrin Complexes to Enhance their Solubility and Dissolution Parameters

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ABSTRACT

Itraconazole is a potent triazole antifungal drug which has low solubility at physiological pH conditions. It is active in vitro against a wide variety of fungi with a spectrum of activity which qualitatively resembles that of ketoconazole, the first oral azole to gain widespread acceptance. Itraconazole binds more avidly to fungal cytochrome P-450 than does ketoconazole and unlike ketoconazole, has little effect on mammalian cytochrome P-450 enzyme systems.The purpose of present work was to explore the feasibility and preparation of the Itraconazole Hydrochloride salt to improve the solubility and dissolution rate of poorly soluble drug Itraconazole. Itraconazole Hydrochloride was synthesized by using addition reaction with hydrochloric acid. Then it was incorporated into a new derivative of cyclodextrins Sulfobutyl ether β-Cyclodextrin (CAPTISOL) and 2-Hydroxypropyl β-Cyclodextrin by using physical mixing, kneading and co-evaporation techniques. 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. The results indicated Itraconazole HCL-Captisol (1:2 molar ratio) prepared by kneading method shows better characteristics when compared with pure drug and other formulations.

Keywords: Itraconazole; Itraconazole hydrochloride; Sulfobutyl ether$_7$ β-Cyclodextrin; 2-Hydroxypropyl β-Cyclodextrin; dissolution enhancement.

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INTRODUCTION

Oral delivery is often the most preferred route of drug administration because of several advantages over other routes. But majorly novel innovative drug candidates suffer from poor pharmacokinetics and this reduces bioavailability which mostly seen in oral drug delivery[1]. The reasons for poor pharmacokinetics are usually related to their poor solubility. Therefore, the improvement of drug solubility thereby its oral bio-availability remains one of most challenging aspects of drug development process to scientists in the pharmaceutical industry[2]. Biopharmaceutics Classification System (BCS) class II poorly water soluble compounds mainly exhibits bioavailability which is relative to the dissolution rate of the drug product, with increased dissolution rates will provide improved oral bioavailability[3-4]. It has been reported that 40 – 70% of new chemical entities (NCEs) in development are classified as BCS class II compounds, having mainly solubility issues which limits oral bioavailability[5- 6]. Out of these compounds, it has been estimated 15 – 30% can exhibit some improvement in dissolution rate through modification of the drug crystal structure[7]. Kaplan has suggested that the solubility of a drug more than 10mg/mL at a pH less than 7 is expected to have no dissolution as well as bioavailability related problems but drugs whose solubility is below 1mg/mL there will be dissolution as well as bioavailability problems[8]. Solubility of a drug is an intrinsic property and it can be changed by chemical modification of the molecule and also by salt formation or prodrug formation[9-12].

Salt formation changes the pharmacokinetic properties of a drug by modifying its physical and chemical properties. The type of salt to be prepared depends on the chemical properties (pKa, logP) of the drug, counter ion, mode of preparation, safety of the counter ion and route of administration. The common criteria for selection of counter ion was proposed by Tong and Whitesell[13] which claims mainly for preparation of salts from a basic drug, the pKa of salt should be at least 2 pH units lower than the pKa of the drug. For weakly basic drugs, a salt of an inorganic acid (e.g., hydrochloride, sulfate, or phosphate) or sulfonic acid (mesylate, besylate or isothionate) should be selected[14]. Hydrochlorides are the most commercially marketed salts (nearly 44%) which were previously compiled from the 1993 edition of Martindale, the Extra Pharmacopeia[15-16]. Hydrochloride salts are common because of their low molecular weight and low toxicity.

Cyclodextrins (CDs) are useful functional excipients that have enjoyed 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[17-18]. The formation of inclusion complexes provides numerous advantages in pharmaceutical formulation development β-CD were reported to increase bioavailability of poorly soluble drugs by increasing the drug solubility[19]. Hydroxypropyl betacyclodextrins[20] are modified β CDs having a higher aqueous solubility (above 60%) at room temperature. They are used as a solubilizer for hydrophobic molecules in oral liquids, oral solids, parenterals, pressurized metered dose inhalers, dry powder inhalers and also in topical formulations[21-25]. It can also act as a stabilizer during processing and storage of formulations[26-27]. The reported advantage of hydroxyl propyl beta Cyclodextrin over unsubstituted beta Cyclodextrin is its greater water solubility[28]. Sulfobutyl ether β-Cyclodextrin (SBE_7-β-CD) [Captisol®][29-30] is a chemically modified β- cyclodextrins that is a cyclic hydrophilic oligosaccharide which is negatively charged in aqueous media. The solubility of Captisol (70 g/100 ml at 25 °C) in water is significantly higher than the parent β-Cyclodextrin (1.85 g/100 ml at 25 °C). It does not exhibit the nephrotoxicity and cytotoxicity which is generally associated with other β-CDs[31-33]. Some of the investigations also reported that the drug inclusion complex with Sulfo Butyl $Ether_7$ β-Cyclodextrin provided a protective effect against drug-induced cytotoxicity[34]. Based on these advantages, Captisol has been selected to study the effect of improving the physiochemical properties of poorly water-soluble drug itraconazole.

Itraconazole[35] is a potent triazole antifungal drug which has low solubility at physiological pH conditions. It is active in vitro against a wide variety of fungi with a spectrum of activity which qualitatively resembles that of ketoconazole, the first oral azole to gain widespread acceptance. Itraconazole binds more avidly to fungal cytochrome P-450 than ketoconazole and unlike ketoconazole, has little effect on mammalian cytochrome P-450 enzyme systems. Itraconazole (ITZ) is used in the treatment of both local and systemic fungal infections. ITR has the characteristic of pH dependent solubility having highest solubility at acidic side (4µg/ml) compared to basic pH (1µg/ml). However, because of highly liphophilic nature (log P= 6.2) it can easily penetrate into intestinal membrane. Various techniques have been reported for enhancing the solubility and bioavailability of itraconazole, but the salt formation[36] and inclusion complexes[37] showed some promising results. Keeping these in the view the present work has planned with an objective to synthesize Itraconazole hydrochloride salt form from Itraconazole. Further this salt form have studied for improvement of solubility and dissolution by preparing inclusion complexes with Sulfobutyl$_7$ Ether β-Cyclodextrin (Captisol®) and 2-HP β-Cyclodextrin 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 and dissolution studies.

MATERIALS AND METHODS

Chemicals:

Itraconazole was a gift sample obtained from Pharmatech, Hyderabad. Sulfobutyl$_7$ Ether β-Cyclodextrin (Captisol®) (average molecular weight 2163 and degree of substitution 6.5) was obtained from Cydex laboratories and 2-Hydroxypropyl β-Cyclodextrin (average molecular weight 1400) from sigma Aldrich. Hydrochloric acid (A.R. grade), methanol (A.R grade) and Di-chloro methane (A.R. grade) were purchased from Merck. All other chemicals used in this study were of analytical grade.

Preparation of Itraconazole Salts:

Itraconazole salt was synthesized from a modified method by using acid addition reaction method[38-40]. Itraconazole pure drug was accurately weighed about 5g (5.4 mmol) and suspended in about 10 ml of dichloromethane. To this solution about 400 mg of Concentrated Hydrochloric acid (11.42 mmol) was added and dissolved. The above suspension heated at 50^0 C for 1hr under reflux using rotary evaporator. After one hour 700 mpa vacuum was applied and continued reflex for 1 more hr to form a precipitate of salt. The mixture was allowed to stand overnight at room temperature and the precipitated product was collected and dried at 60°C for 1 hour and finally shifted through #100 mesh sieve. The final product was stored in an air tight container and then placed in desiccators.

Solubility Studies:

Solubility studies for pure ITR and ITR HCL 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 37^0C 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[41].

Phase Solubility Studies:

A phase solubility[42-43] study was carried out to investigate the effect of Sulfobutyl ether β-Cyclodextrin & 2-Hydroxypropyl β-CD on the solubility of Itraconazole & Itraconazole Hydrochloride using the method reported by Higuchi and Connors. Sulfobutyl ether β-Cyclodextrin and 2HP β-CD were added separately in different conical flasks containing simulated gastric fluid (pH 1.2 or 0.1N HCL) to obtain concentrations of 5, 10, 20, 40 and 80

mM. To each of the above solutions excess amounts of Itraconazole and Itraconazole HCL were added separately and then shaken using test tube 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 Itraconazole and Itraconazole HCL at 258 nm using UV-Visible spectrophotometer. A graph was plotted between Itraconazole concentrations (in mM) and Itraconazole HCL concentrations (in mM) against the concentration of Sulfobutyl ether β-Cyclodextrin (in mM) and 2HP β-CD (in mM). The stability constant for the complex was determined from the graph using the following (Eq.1).

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

Where slope was obtained from the graph and S_0 was the equilibrium solubility of Itraconazole and Itraconazole HCL in 0.1 N HCl.

Preparation of Inclusion Complexes:

The inclusion complexes of ITR with both Sulfobutyl ether β-CD & 2HP β-CD (1:2 & 1:3) and similarly ITRHCL with Sulfobutyl ether β-CD & 2HP β-CD (1:2 & 1:3) were prepared by using physical mixing, kneading and co-evaporation technique[44]. Physical mixtures were prepared by simple mixing in a mortar with pestle for 10 min. The powders of both ITR, ITR HCL with Sulfobutyl ether β-CD and 2HP β-CD 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 both ITR, ITR HCL with SBE_7 β-CD and 2HP β-CD 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 Sulfobutyl ether β-CD and 2HP β-CD were added to the solution of ITR and ITR HCL in a solvent blend of methanol: dichloromethane at a volume ratio of 2:3. The resultant mixture was stirred for nearly 1 hr under mechanical stirrer until solvent gets evaporated to obtain wet mass and dried in an oven at 55 °C until it get dry. The dried mass was pulverized and sifted through #100 sieve.

Fourier Infra Red Spectroscopy (FTIR):

Fourier transform infrared spectroscopy (FTIR) spectra of ITR, ITR HCL, SBE_7 β-CD, 2HP β-CD, ITR/SBE7 β-CD complexes, ITR/2HP β-CD complexes, ITR HCL/SBE7 β-CD complexes and ITR HCL/2HP β-CD complexes were recorded using a Fourier Transform Infrared

spectrophotometer (Perkin Elmer, Spectrum Two). Samples were prepared using KBr (Spectroscopic grade) disks by means of hydraulic pellet press at a pressure. The samples were scanned from 400–4000 cm^{-1} with a resolution of 4 cm^{-1}

X-ray diffraction analysis:

Powder X-ray diffraction (XRD) patterns of ITR, ITR HCL, SBE_7 β-CD, 2HP β-CD, ITR/SBE7 β-CD complexes, ITR/2HP β-CD complexes, ITR HCL/SBE7 β-CD complexes and ITR HCL/2HP β-CD complexes were recorded on a PAN Analytical X'Pert powder X-ray diffractometer (X-Perto PRO) using Cu Kα radiation, Ni-filtered, a voltage of 40 kV and 60 mA current. The rate of scanning was 4°/min over the diffraction angle range (2θ) of 3–50°.

Drug Content Estimation:

Accurate weighed 50 mg of the sample was taken and transferred in to 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 µm filter and suitable diluted for analysis of the drug content 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[45] 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±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 diluted suitably 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® was also evaluated for dissolution to compare with ITR and prepared ITR salts inclusion complexes. All the dissolution experiments were conducted in triplicate and the mean values are reported. The dissolution efficiency (DE_{90}), difference factor (f_1) and similarity factor (f_2) was calculated for itraconazole and its salt complexes[46]. The kinetics of ITR release from complexes was studied by subjecting the dissolution data to zero order and first order kinetics and mechanism of drug release was studied by subjecting the dissolution data to Hixson Crowell, Korsmeyer Peppas and Higuchi diffusion mechanisms[47-48].

RESULTS AND DISCUSSION

Preparation of Itraconazole Salt Forms:

The salt form of Itraconazole Hydrochloride synthesized was obtained as a pale white powder with a practical yield of 90%. The chemical reaction[39] shown in Figure 1 below was drawn using Chem draw and the prepared salt form was stored in an air tight container and placed in desiccator.

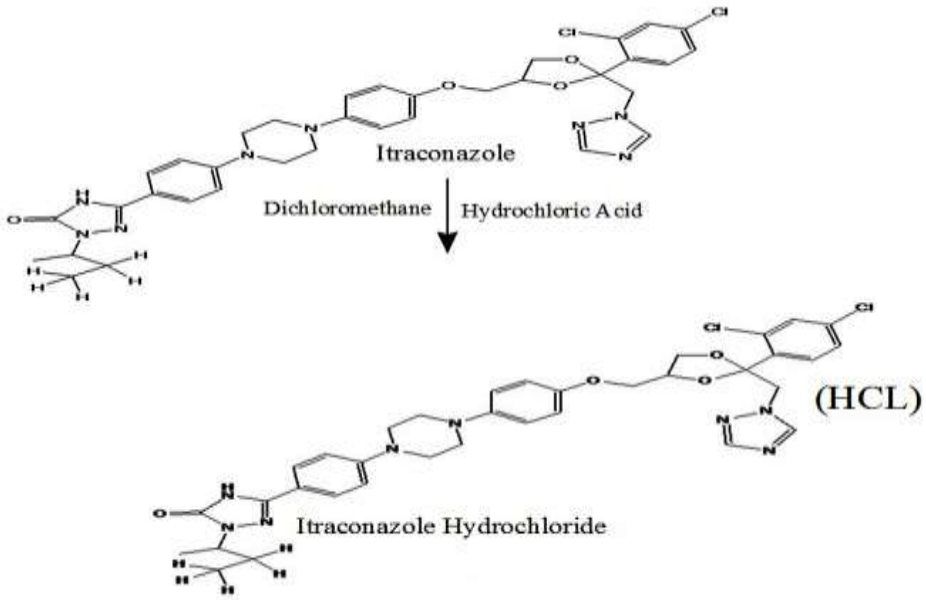

Figure 1: Synthesis of Itraconazole hydrochloride from Itraconazole

Solubility Studies of Salt Forms and Itraconazole:

The results show that in water the solubility of Itraconazole was only 2.388µg/ml at 37^0 C and in simulated gastric fluid the solubility was 11.59µg/ml. After conversion of salt form the solubility of Itraconazole HCL in water was increased from 2.388µg/ml to 23.86µg/ml at 37^0 C and in simulated gastric fluid the solubility was increased from 11.59µg/ml to 93.60µg/ml. Itraconazole salt form was 8 times more soluble than its base.

Phase Solubility Studies:

The effects of cyclodextrins on the aqueous solubility of ITR & ITR HCL were evaluated using the phase solubility method. The results indicated (as shown in Table 1 below) that there is a linear relationship between the increase in ITR & ITR HCL solubility on increase of SBE_7 β-CD & 2 HP-β-CD concentrations which characterizes the complexation of the drug. 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). The slope value was less than one i.e., for Itraconazole it is 0.034 and for Itraconazole Hydrochloride it was

0.035 respectively. 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 apparent Stability Constant (K_S) obtained from the slope of linear portion of phase solubility diagrams (as shown in Figure 2a to Figure 2d) for ITR with SBE7 β-CD, ITR with 2HP β-CD, ITR HCL with SBE7 β-CD and ITR HCL with 2HP β-CD were found to be 79.68M^{-1} , 66.40 M^{-1}, 254.73 M^{-1} and 223.95 M^{-1} respectively.

Table 1: Phase Solubility Data

S. No	Concentration of SBE_7 β-CD & 2HP β-CD (mM)	Concentration of ITR with 2HP β-CD (mM)	Concentration of ITR with SBE_7 β-CD (mM)	Concentration of ITR HCL with 2HP β-CD (mM)	Concentration of ITR HCL with SBE_7 β-CD (mM)
1	0	0.00005	0.00006	0.00019	0.00020
2	5	0.0312	0.0302	0.359	0.321
3	10	0.0493	0.0459	0.550	0.536
4	20	0.134	0.122	1.587	1.437
5	40	0.221	0.199	2.589	2.253
6	80	0.372	0.325	4.087	3.819
Stability Constant (k_s)		**79.68M^{-1}**	**66.40M^{-1}**	**254.73M^{-1}**	**223.95M^{-1}**

mM – Milli molar

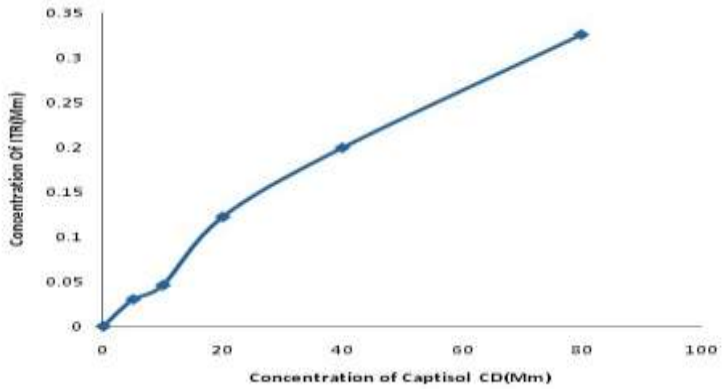

Figure 2a: Phase Solubility Graph of Itraconazole with SBE_7 β-CD

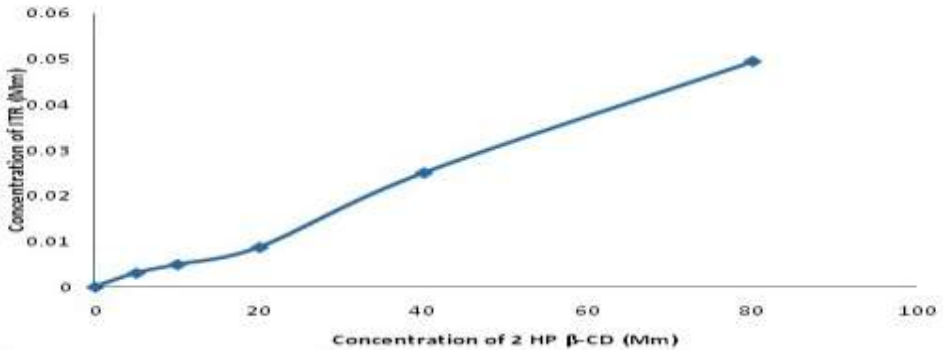

Figure 2b: Phase Solubility Graph of Itraconazole with 2HP β-CD

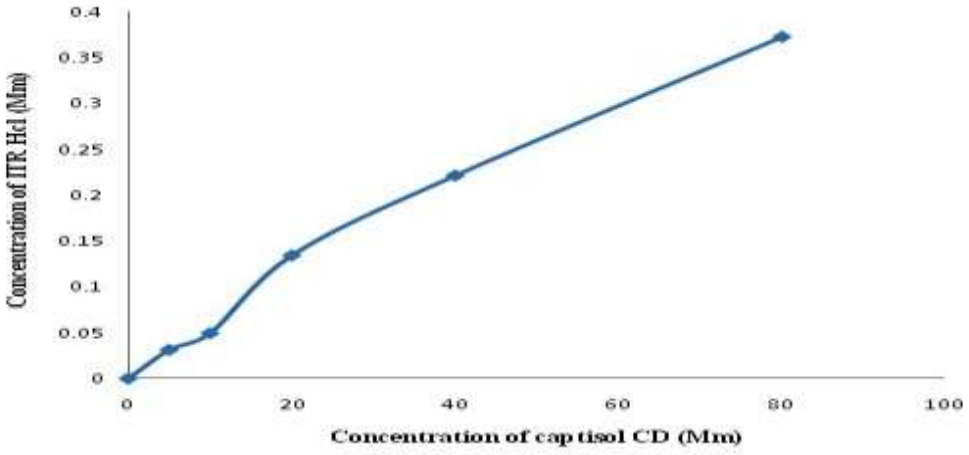

Figure 2c: Phase Solubility Graph of Itraconazole hydrochloride with SBE_7 β-CD

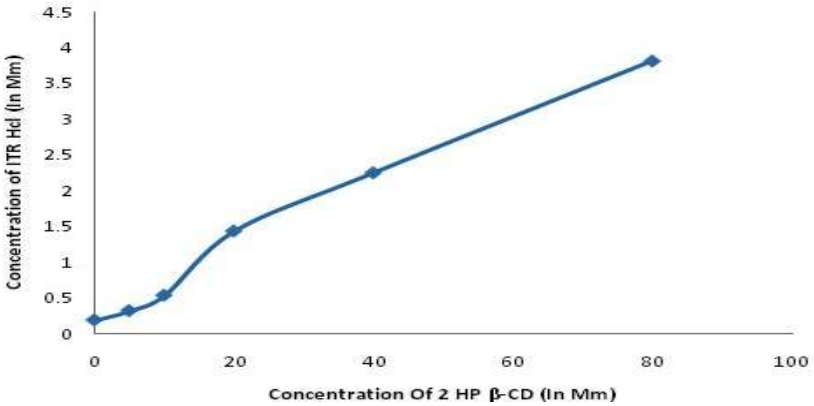

Figure 2d: Phase Solubility Graph of Itraconazole hydrochloride with 2HP β-CD

Infra red spectroscopy (IR):

FTIR results suggested that there are significant changes observed in characteristic bands and functional groups of spectra for ITR HCL: Captisol (1:2 molar ratio) and ITR HCL: 2HP-β CD (1:2 molar ratio) prepared by Kneading method, that indicates there is a complex formation. FTIR spectrums were shown below in Figure 3, 4 & 5. The IR spectrum of the pure drug indicated the presence of characteristic peaks of carboxylate group (o-c-o) in the range of 1550-1660cm $^{-1}$, C-N stretch from 1073cm $^{-1}$, Chlorine group at 700-850cm^{-1} and Benzene moiety from 3100-300cm^{-1}. FTIR studies revealed that Itraconazole HCL showed two typical bands at 3369 and 3283 cm $^{-1}$ due to N-H primary stretching vibration and a band at 3170 cm $^{-1}$ due to N-H secondary stretching and characteristics bands at 1623 and 1560 cm $^{-1}$ assigned to C=N stretching. FTIR results with Complexation suggested that there is no significant chemical interaction between the drug and the Cyclodextrin for complexed products, which confirms the stability of drug in the powdered form.

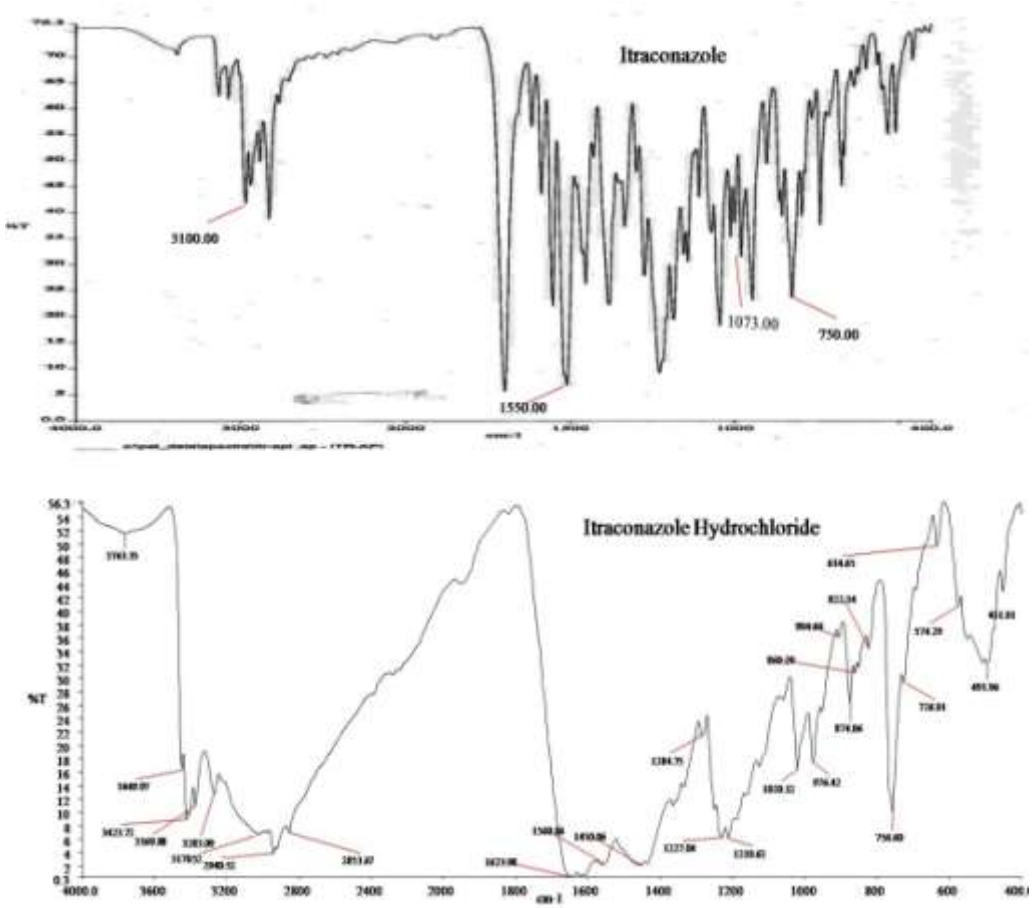

Figure 3: IR spectra of ITR (Pure Drug) and ITR HCL

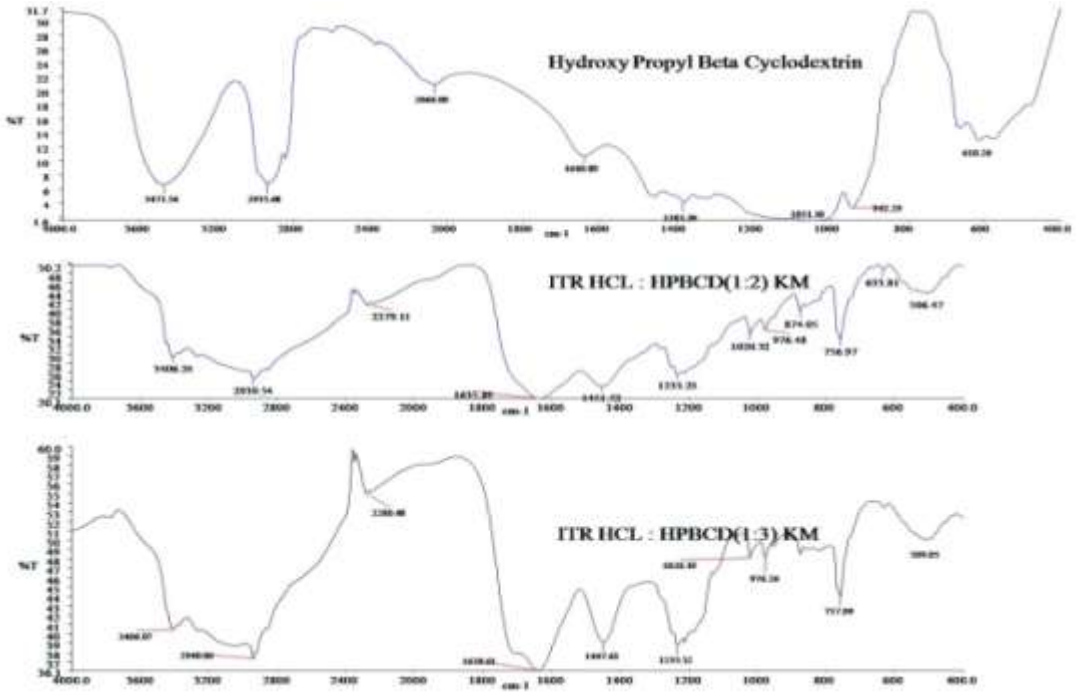

Figure 4: IR spectra of 2-HP β-CD, ITR HCL-2HP β-CD-KN (1:2) and ITR HCL-2HP β-CD-KN (1:3)

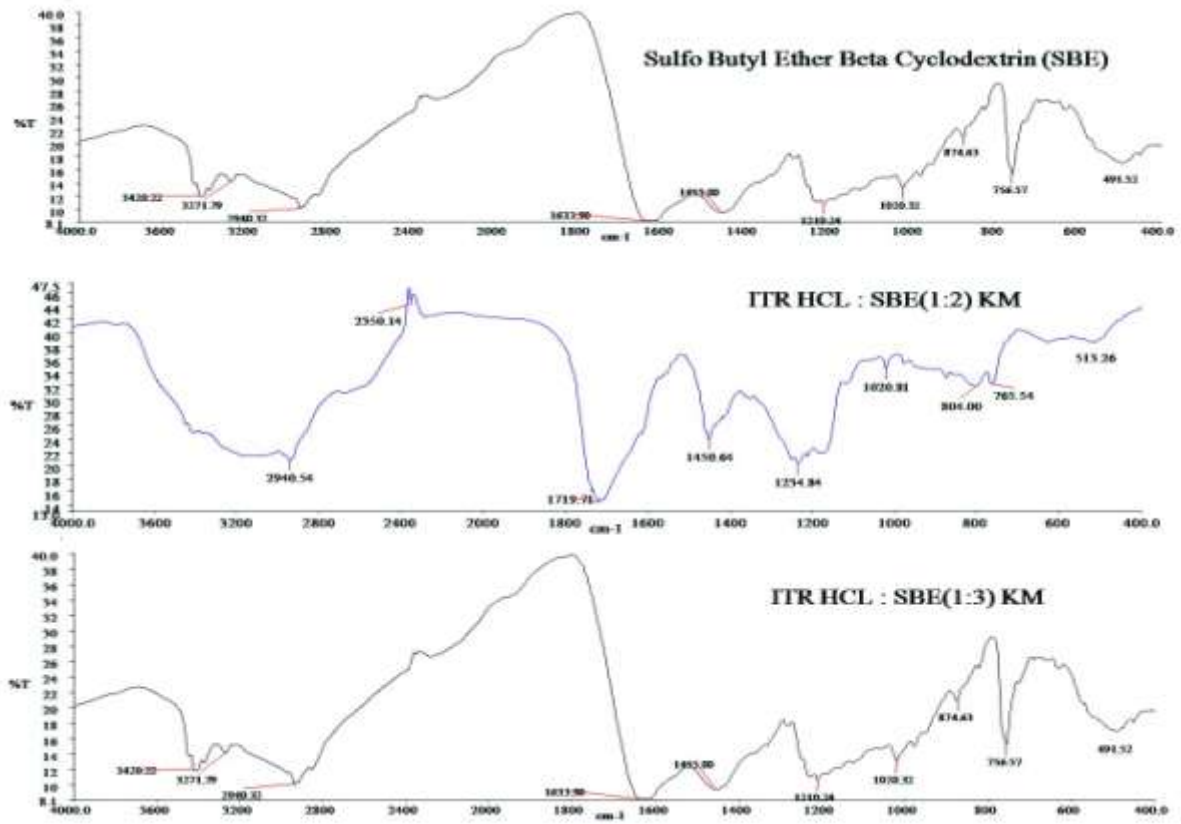

Figure 5: IR spectra of Captisol (SBE_7 β-CD), ITR HCL- SBE_7 β-CD –KN and ITR HCL- SBE_7 β-CD –KN (1:3)

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 samples are shown in Figure 6 and Peaks were intense and sharp, indicating its crystalline nature. However, the characteristic crystalline peak of ITR was also observed for the complexes, but the peak size was reduced which indicates that intensity of crystallinity was decreased when compared with the pure drug itself. The XRD patterns of salt complexed products have been found to have less intensity of crystalline peaks when compared with the pure drug complexed patterns.

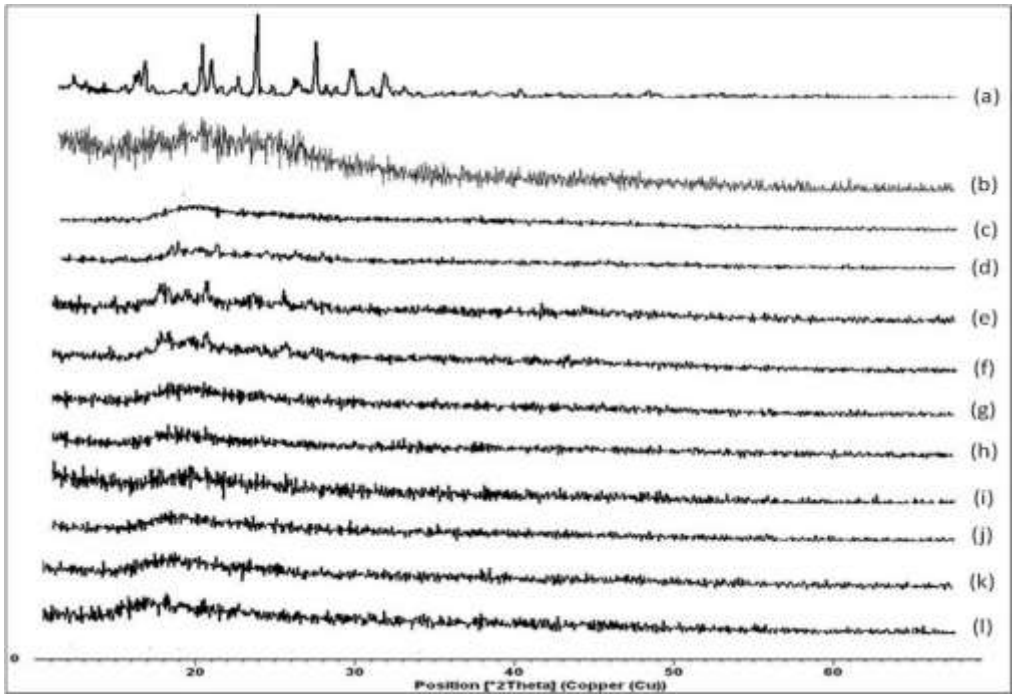

Figure 6 XRD Patterns of (a)ITR (b)ITR HCL (c)Cap (d)2HP βCD (e)ITR-2HP βCD(1:2) (f)ITR-2HP βCD(1:3) (g)ITR-Cap(1:2) (h)ITR-Cap(1:3) (i) ITR HCL-2HP β-CD(1:2) (j)ITR HCL-2HP βCD(1:3) (k)ITR HCL-Cap(1:2) (l)ITR HCL- Cap (1:3)

Drug Content Estimation:

All the drug content values (as shown in Table 2) of Itraconazole complexed products were found to be in the range of 85.66% to 99.64%. The highest drug content was found to be 99.64% for kneading ITR HCL: Captisol (1:2 molar ratio) solid complex, which indicating preparation of solid complexes with kneading method has better content uniformity when compared with other complexed products.

Table 2: Different Itraconazole complexes and their drug content

Complexes	Method	Terminology	Drug content (% w/w)	
			1:2	1:3
ITR + 2HP β-CD	Kneading	ITR-2HP β-CD-KN	89.74±0.12	88.56±0.26
	Co-Evaporation	ITR- 2HP β-CD -EV	90.12±0.52	90.58±0.25
	Physical Mixture	ITR- 2HP β-CD -PM	82.89±0.39	81.12±0.19
ITR + Captisol (SBE_7 β-CD)	Kneading	ITR- SBE_7 β-CD -KN	91.23±0.44	90.23±0.41
	Co-Evaporation	ITR- SBE_7 β-CD -EV	93.83±0.56	91.36±0.51
	Physical Mixture	ITR- SBE_7 β-CD -PM	85.89±0.33	85.66±0.35
ITR HCL + 2HP β-CD	Kneading	ITR HCL-2HP β-CD-KN	99.64±0.22	96.64±0.28
	Co-Evaporation	ITR HCL-2HP β-CD -EV	95.55±0.29	94.55±0.27
	Physical Mixture	ITR HCL-2HP β-CD -PM	90.99±0.36	87.94±0.33
ITR HCL +Captisol (SBE_7 β-CD)	Kneading	ITR HCL-SBE_7 β-CD-KN	98.45±0.58	93.86±0.52
	Co-Evaporation	ITR HCL- SBE_7 β-CD -EV	94.88±0.21	91.39±0.24
	Physical Mixture	ITR HCL- SBE_7 β-CD-PM	89.04±0.41	90.11±0.45

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 are shown in Figure 7a, Figure 7b and Figure 7c respectively. Itraconazole pure drug has dissolved only 16.28 % in 90 minutes indicating the poor solubility and thereby dissolution.

In physical mixture complexes the percentage drug release of 1:2 & 1:3 molar ratios of ITR: Captisol in 90 minutes is only 17.12 % & 22.62 % w/w and for ITR: 2HP-β CD percentage drug release is 17.26 % & 21.76% w/w respectively. Kneading method complexes showed percentage drug release of 39.16 % & 37.12 % w/w in 1:2 & 1:3 molar ratios of ITR: Captisol and 37.54 %, 35.21 % w/w drug release for 1:2 & 1:3 molar ratios of ITR: 2HP-β CD. Co-Evaporation method complexes showed dissolution percentage release of 37.29 % and 36.21 % w/w for 1:2 and 1:3 molar ratios of ITR: Captisol and in similar manner for 1:2 and 1:3 molar ratios of ITR: 2HP-β CD percentage drug release was found to be 36.28% and 35.12% w/w respectively. The data indicated that the pure drug complexes with Captisol and 2HP β-CD could not be able to increase the dissolution to the required level.

The dissolution of 1:2 and 1:3 molar ratio of ITR HCL: Captisol physical mixture complex in 90 minutes is 50.12 % and 44.19 % w/w and for 1:2 and 1:3 molar ratios of ITR HCL: 2HP-β CD is 48.78% w/w and 42.15% w/w respectively. Kneading method complexes showed 99.10 % and 89.72 % w/w drug release in 90 min for 1:2 and 1:3 molar ratios of ITR HCL: Captisol and 96.22 % w/w and 85.56 % w/w for 1:2 and 1:3 molar ratios of ITR HCL: 2HP-β CD. Co-Evaporation method complexes showed dissolution percentage release of 73.96 % and 70.68 % w/w for 1:2 and 1:3 molar ratios of ITR HCL: Captisol and in similar manner for 1:2 and 1:3 molar ratios of ITR HCL: 2HP-β CD percentage drug release was found to be 71.26% and 70.28% w/w respectively. This data more clearly indicated that the itraconazole hydrochloride salt complexes with Captisol and 2HP β-CD could more significantly increase the dissolution rate to the required level than itraconazole alone. For comparison the dissolution of commercial Sporanox capsules also performed which showed only 95.38 % ITR release in 90 minutes (as shown in Figure 7d).

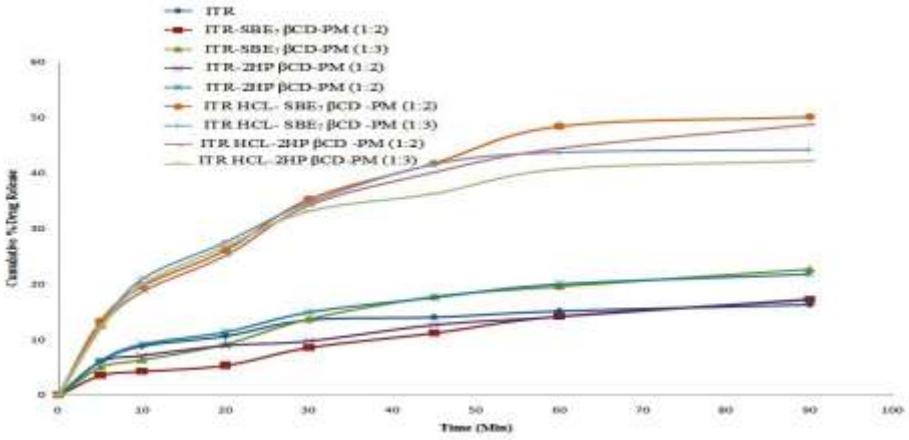

Figure 7a: Percentage drug release of complexes prepared by PM Method

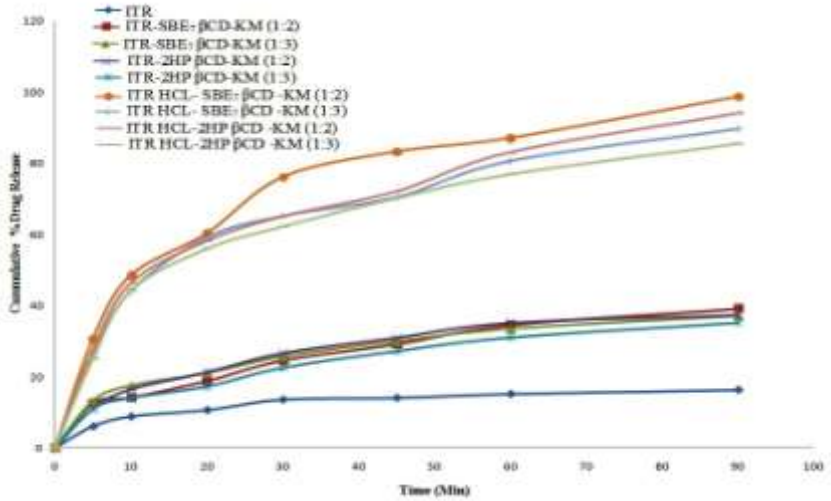

Figure 7b: Percentage drug release of complexes prepared by Kneading Method

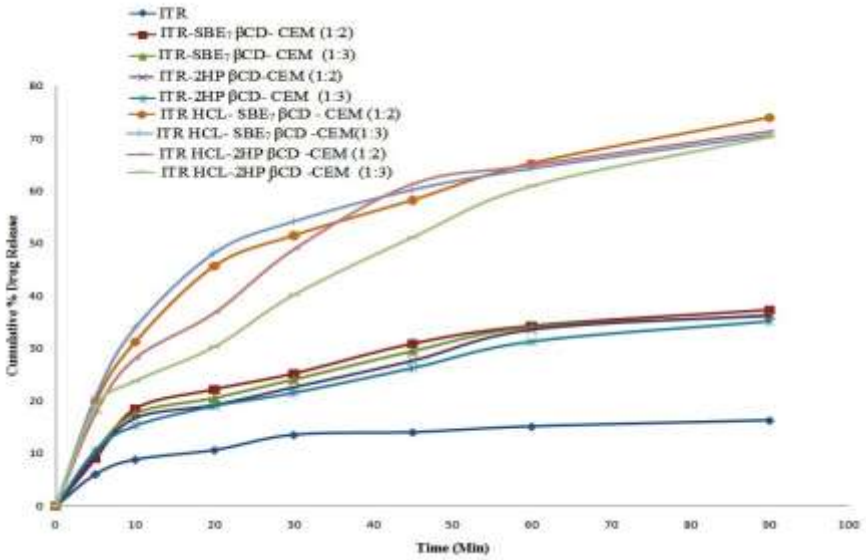

Figure 7c: Percentage drug release of complexes prepared by Co-Evap Method

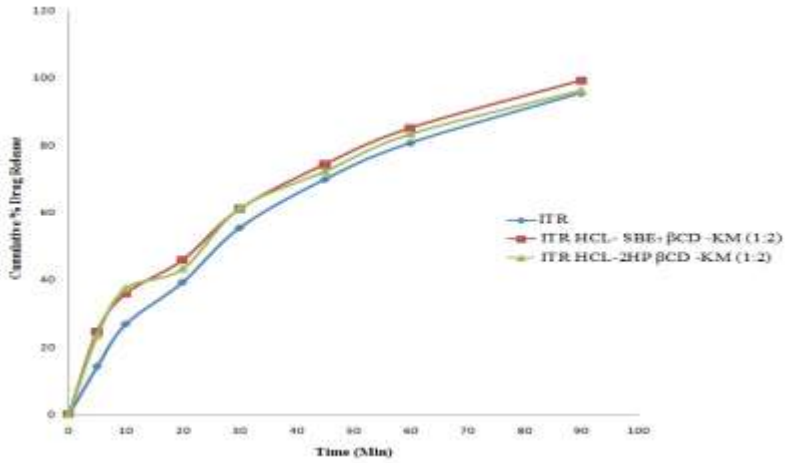

Figure 7d: Percentage drug release of complexes with Sporanox®

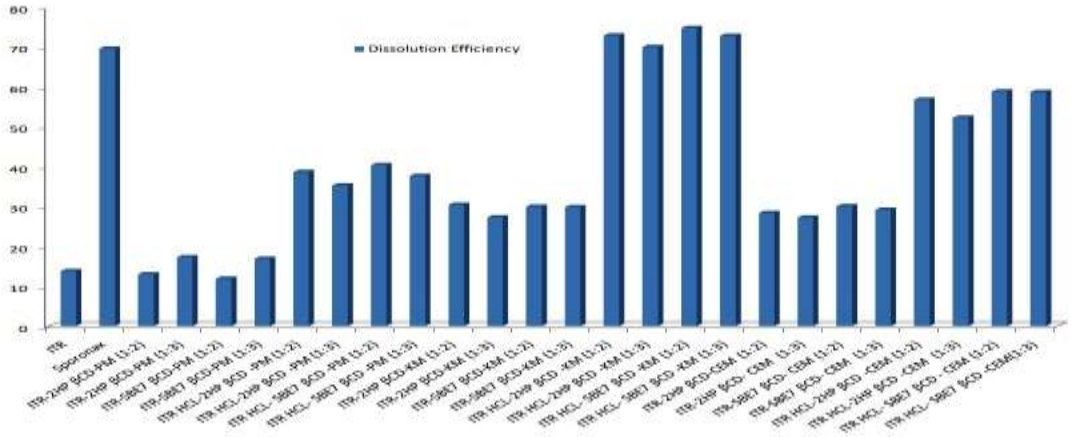

Figure 8: Dissolution Efficiency (DE_{90} %) of Itraconazole and Itraconazole salt complexes with PM, KM and EV methods

The dissolution efficiency (DE_{90}) at 90 minutes was calculated and the values shown in Table 3 and Figure 8. Pure itraconazole showed DE_{90} 13.79%, ITR HCL: Captisol (1:2 weight ratio) complexes prepared by kneading showed 74.80% and ITR HCL: 2HP-β CD (1:2 weight ratio) complexes prepared by kneading showed 72.96%. DE_{90} of Sporonax capsules is 69.63% and the data indicates that the itraconazole hydrochloride salt complexes with Captisol and 2HP-β CD prepared by kneading technique are efficient in improving the dissolution of ITR and are comparable with commercial Sporonax capsules. The difference factor (f_1) and similarity factor (f_2) values of ITR HCL: Captisol (1:2 molar ratio) prepared by kneading method showed 9 and 62 (Shown in Table 3). In the similar manner difference factor (f_1) and similarity factor (f_2) values of ITR HCL: 2 HP β-CD (1:2 molar ratio) prepared by kneading method showed 12 and 60. These values also indicated that there is equivalence of dissolution profile for ITR HCL: Captisol (1:2 molar ratio) and ITR HCL: 2 HP β-CD (1:2 molar ratio) prepared by kneading

method to that of commercial capsules Sporanox®. The kinetics of ITR release from complexes was studied by subjecting the dissolution data to zero order, first order kinetics (as shown in Table 4 below). The results indicated that the drug release follows first order kinetics and the mechanism of drug release was found to be by diffusion.

Table 3: Dissolution parameters of Itraconazole

Sample	Dissolution Efficiency(DE_{90} %)	Difference Factor(f_1)	Similarity Factor(f_2)
ITR	13.79	78	17
Sporonax	69.63	-	-
ITR-2HP βCD-PM (1:2)	13.00	80	17
ITR-2HP βCD-PM (1:3)	17.26	74	18
ITR-SBE_7 βCD-PM (1:2)	11.89	83	16
ITR-SBE_7 βCD-PM (1:3)	16.90	75	18
ITR HCL-2HP βCD -PM (1:2)	38.67	41	30
ITR HCL-2HP βCD -PM (1:3)	35.29	45	28
ITR HCL- SBE_7 βCD -PM (1:2)	40.38	38	31
ITR HCL- SBE_7 βCD -PM (1:3)	37.69	41	29
ITR-2HP βCD-KM (1:2)	30.44	53	25
ITR-2HP βCD-KM (1:3)	27.26	58	23
ITR-SBE_7 βCD-KM (1:2)	29.96	55	24
ITR-SBE_7 βCD-KM (1:3)	29.86	53	24
ITR HCL-2HP βCD -KM (1:2)	72.96	12	60
ITR HCL-2HP βCD -KM (1:3)	70.04	17	49
ITR HCL- SBE_7 βCD -KM (1:2)	74.80	9	62
ITR HCL- SBE_7 βCD -KM (1:3)	72.86	17	48
ITR-2HP βCD-CEM (1:2)	28.38	57	24
ITR-2HP βCD- CEM (1:3)	27.23	58	23
ITR-SBE_7 βCD- CEM (1:2)	30.08	53	25
ITR-SBE_7 βCD- CEM (1:3)	29.20	55	24
ITR HCL-2HP βCD -CEM (1:2)	56.83	16	48
ITR HCL-2HP βCD -CEM (1:3)	52.31	25	42
ITR HCL- SBE_7 βCD - CEM (1:2)	58.88	18	48
ITR HCL- SBE_7 βCD -CEM(1:3)	58.75	20	46

Table 4: Dissolution kinetics of Itraconazole

Sample	Zero order	First order	Hixson Crowell	Korsmeyer Peppas	Higuchi	First order rate constant (k_1)
ITR	0.707	0.728	0.721	0.961	0.925	0.002
Sporonax	0.923	0.976	0.994	0.987	0.990	2.388
ITR-2HP βCD-PM (1:2)	0.867	0.888	0.881	0.975	0.983	0.002
ITR-2HP βCD-PM (1:3)	0.840	0.864	0.856	0.988	0.984	0.002
ITR-SBE_7 βCD-PM (1:2)	0.959	0.967	0.967	0.956	0.971	0.002
ITR-SBE_7 βCD-PM (1:3)	0.907	0.924	0.919	0.980	0.984	0.004

ITR HCL-2HP βCD-PM (1:2)	0.841	0.898	0.881	0.984	0.981	0.006
ITR HCL-2HP βCD-PM (1:3)	0.759	0.815	0.797	0.955	0.951	0.004
ITR HCL- SBE_7 βCD -PM (1:2)	0.834	0.889	0.872	0.983	0.976	0.006
ITR HCL- SBE_7 βCD -PM (1:3)	0.749	0.800	0.784	0.946	0.941	0.004
ITR-2HP βCD-KM (1:2)	0.812	0.860	0.845	0.986	0.975	0.861
ITR-2HP βCD-KM (1:3)	0.864	0.906	0.893	0.989	0.990	0.799
ITR-SBE_7 βCD-KM (1:2)	0.879	0.923	0.909	0.977	0.991	0.895
ITR-SBE_7 βCD-KM (1:3)	0.804	0.858	0.841	0.955	0.973	0.806
ITR HCL-2HP βCD -KM (1:2)	0.883	0.973	0.989	0.984	0.991	2.247
ITR HCL-2HP βCD -KM (1:3)	0.757	0.948	0.895	0.942	0.950	1.844
ITR HCL- SBE_7 βCD -KM (1:2)	0.893	0.918	0.986	0.995	0.997	2.321
ITR HCL- SBE_7 βCD -KM (1:3)	0.766	0.965	0.917	0.952	0.954	1.934
ITR-2HP βCD-CEM (1:2)	0847	0.891	0.877	0.968	0.981	0.829
ITR-2HP βCD- CEM (1:3)	0.854	0.899	0.855	0.991	0.987	0.783
ITR-SBE_7 βCD- CEM (1:2)	0.787	0.847	0.832	0.933	0.964	0.847
ITR-SBE_7 βCD- CEM (1:3)	0.812	0.859	0.844	0.970	0.972	0.819
ITR HCL-2HP βCD -CEM (1:2)	0.836	0.931	0.904	0.980	0.978	1.699
ITR HCL-2HP βCD -CEM (1:3)	0.911	0.981	0.964	0.983	0.993	1.655
ITR HCL- SBE_7 βCD - CEM (1:2)	0.810	0.937	0.900	0.973	0.974	1.658
ITR HCL- SBE_7 βCD -CEM(1:3)	0.743	0.873	0.832	0.946	0.943	1.547

The significant enhancement in dissolution rate of Itraconazole salt form with their SBE_7 βCD (Captisol) and 2 HP β-CD complexation mixtures could be explicated by several ways like reduction in particle size of ITR after salt formation into ITR hydrochloride, as the smaller sized particles expose more surface area of the powder in water resulting into better wetting and solubility. Low crystalline nature of the salt was shown by X-ray diffraction patterns, as low or non crystalline states of a molecule are the higher energy states and therefore usually have better solubility than that of the corresponding high crystalline forms. Phase solubility studies also showed the effect of Cyclodextrin on the aqueous solubility of ITR and ITR HCL. In addition to this inclusion complexation of ITR HCL with SBE_7 βCD (Captisol) and 2HP-β CD in presence of an aqueous environment can be another major reason for amplification in dissolution rate. This effect is more prominent in case of kneading method mixtures of ITR HCL with SBE_7 βCD (Captisol) in 1:2 molar ratios because for better aqueous solubility of SBE_7 βCD (Captisol) than other cyclodextrins. The dissolution profile exhibited that faster dissolution for ITR HCL with SBE_7 βCD (1:2 molar ratio) in kneading method when compared with ITR HCL: 2HP-β CD (1:2 molar ratio) and the marketed capsules Sporonax®.

CONCLUSION

Based on present study and results it is concluded that formation of hydrochloride salt form could significantly improve the solubility and dissolution rate of Itraconazole. These results also

suggested that an Itraconazole Hydrochloride Complex of sulfo derivative cyclodextrin (Captisol) prepared by Kneading technique has greater solubility and dissolution rate when compared with pure drug Itraconazole and commercial Sporanox® capsules.

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