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Enhancement of dissolution amount and *in vivo* bioavailability of itraconazole by complexation with β-cyclodextrin using supercritical carbon dioxide

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Dedicated to the memory of Dr. Hazem A. Hassan who passed away prior to publication of this paper.

Abstract

The main objective of this study was to improve the inclusion formation between itraconazole and β-cyclodextrin and thus enhance dissolution amount and bioavailability characteristics of itraconazole. Inclusion complexes between itraconazole and β-cyclodextrin were prepared using simple physical mixing, conventional coprecipitation method, and supercritical carbon dioxide (SC CO_2). Effects of process variables (temperature, pressure) and drug:cyclodextrin ratio on inclusion yield and thermal behavior of the solid complexes prepared by SC CO_2 were studied and compared to those obtained by physical mixing and coprecipitation methods. In addition, dissolution amounts of the products obtained by different methods were measured in gastric fluid. Finally, pharmacokinetic studies of the inclusion complexes were conducted in male Wistar rats to assess the bioavailability of the prepared complexes.

Results showed that temperature, pressure and itraconazole:β-cyclodextrin ratio had significant effects on the inclusion yield of the complex prepared by SC CO_2 method. Higher inclusion yields were obtained in the SC CO_2 method as compared to physical mixing and coprecipitation methods. *In vivo* drug pharmacokinetic studies showed that the itraconazole-β-cyclodextrin product prepared using SC CO_2 gave higher bioavailability of itraconazole (in blood, liver and kidney of male Wistar rats) as compared to the products obtained by physical mixing or coprecipitation methods.

Keywords: Bioavailability; β-Cyclodextrin; Dissolution; Inclusion complex; Itraconazole; Supercritical carbon dioxide

1. Introduction

Treatment options for coetaneous and systemic fungal infections have been greatly expanded with the advent of the oral azoles such as ketoconazole and fluconazole. These drugs have shown minimal toxicity, while they have demonstrated efficacy against many invasive fungal pathogens. Itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) is an orally active triazole antifungal agent [1]. Itraconazole, which was first synthesized in 1980, demonstrates a broad spectrum of activity against a number of fungal species including dermatophytes, *Malassesia furfur*, *Candida* species, *Aspergillus* species, and *Histoplasma capsulatum* var. *capsulatum* [2]. The mechanism of action of itraconazole appears similar to that of ketoconazole, involving selective disruption of cytochrome P-450 mediated ergosterol synthesis in fungal membranes, thereby leading to cell death [3]. However, itraconazole differs from ketoconazole in that it demonstrates a high degree of lipophilicity and a lack of endocrine-related side effects. Itraconazole is an extremely weak base ($pK_a = 3.7$) which is virtually unionized at physiological pH [4,5]. Drug dissolution is a rate limiting process for the absorption of poorly water-soluble oral drugs such as itraconazole. The

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efficacy of itraconazole can be severely limited by its poor solubility in aqueous solutions. Thus, absorption is enhanced by the concurrent administration of food [6]. Similarly, ketoconazole absorption is markedly inhibited by agents, which increase gastric pH [7].

Improvement of the oral bioavailability of itraconazole has been problematic. However, concentrations of itraconazole, in serum may be variable and suboptimal, thereby potentially compromising therapy. Indeed, treatment failures have been associated with low concentrations of itraconazole in blood in some patients [8]. Because of the hydrophobic structures of all azoles, this may adversely affect concentrations in blood when the drugs are given orally.

Naturally occurring β-cyclodextrin is a cyclic oligosaccharide of seven glucose units which is a product of the enzymatic degradation of starch by *Bacillus macerans*. The physicochemical properties of cyclodextrins are particularly well suited for utilization as carrier molecules of lipophilic drugs. Their structures are analogous to a truncated cone with a hydrophobic interior and hydrophilic exterior. This allows the molecular encapsulation of hydrophobic portions of guest molecules, thus shielding them from the polar forces of aqueous solutions. According to Strickley [9], cyclodextrins interact with some hydrophobic molecules and form a noncovalent inclusion complex that lowers the chemical potential of the molecule in solution and thus enhances the solubility of the molecule. Pharmaceutical modification of drug molecules by inclusion complexation with cyclodextrins (CDs) has been extensively developed to improve solubility, dissolution rate, chemical stability, absorption, and bioavailability of poorly water-soluble drugs, and reduce side effects and toxicity of drugs [10–18]. Studies have shown that cyclodextrins are also able to form non-inclusion complexes. Loftsson et al. [19] discussed numerous reported evidence that cyclodextrins and their complexes can self-associate to form aggregates through non-inclusion complexation or micelle-like structures, which also effectively solubilize poorly water-soluble drugs.

The hydrophobic nature of most azole compounds nominated them as appropriate candidates to be assembled into the cyclodextrin core to increase their solubility. Peeters et al. [20] studied the phase solubility of itraconazole as a function of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and showed that HP-β-CD significantly improves solubility of itraconazole in aqueous solutions. Van Hees et al. [21,22] used SC CO_2 to produce piroxicam-β-cyclodextrin and miconazole–cyclodextrin inclusion complexes and compared their results with conventional methods of physical mixing, spray drying and freeze drying. Charoenchaitrakool et al. [23] showed that ibuprofen-methyl-β-cyclodextrin complexes prepared by passing ibuprofen–laden CO_2 through a methyl-β-cyclodextrin packed bed can enhance dissolution profiles due to the amorphous character and improved wettability of the product. Recently, Türk et al. [24] developed a controlled particle deposition process to prepare ibuprofen-β-cyclodextrin complexes using SC CO_2, which resulted in higher dissolution rates than the physical mixture of ibuprofen and β-cyclodextrin. Several other studies have also focused on the use of supercritical fluids for the preparation of drug–cyclodextrin complexes for enhanced solubility and dissolution rate [25–28]. We have previously characterized itraconazole/β-cyclodextrin products by powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), UV spectrophotometry and differential scanning calorimetry (DSC) and confirmed the formation of inclusion complexes [29,30].

In continuation of our previous work, the present work is undertaken to improve the inclusion formation between itraconazole and β-cyclodextrin and thereby enhance dissolution amount of itraconazole and its bioavailability characteristics. Itraconazole-β-CD inclusion complexes are prepared using SC CO_2, which is a non-toxic, nonhazardous solvent. Properties of supercritical fluids (SCFs) can be changed from gas-like to liquid-like values by simply adjusting the pressure and temperature. Because of these special characteristics, supercritical fluids have received increasing attention in the pharmaceutical field [21,22,25–32].

2. Materials and methods

2.1. Materials

Itraconazole was generously donated by the College of Pharmacy at Oregon State University. β-CD, purchased from Sigma Chemical Co. was subjected to a thermogravimetric (TG) analysis to find the degree of hydration, which was considered in calculation of the concentration. All chemicals were reagent grade (Merck, Darmstadt) and were used without further purification. All solutions were prepared using deionized water (specific conductance $< 2\ \mu S\ cm^{-1}$) and used within 24 h. Bidistilled water was purified by using a Millipore super Q system and was also degassed prior to the preparation of the solutions.

2.2. Preparation of inclusion complexes

Inclusion complexes between itraconazole and β-cyclodextrin (at drug to cyclodextrin molar ratios of 1:2 and 1:4) were prepared by physical mixing, coprecipitation method and SC CO_2 as described earlier [29]. Physical mixtures were prepared by grinding known amounts of itraconazole and β-cyclodextrin powders in a mortar with a pestle. In the coprecipitation method, known amounts of β-cyclodextrin and itraconazole were dissolved in deionized water and methanol, respectively. Both solutions were heated to 65 °C and mixed together. The final solution was continuously mixed at 65 °C to remove the organic solvent after which the mixture was cooled to 5 °C and the crystals were separated by filtration through 0.45 μm membrane filters. The product was dried and kept in a desiccator overnight to remove traces of solvents.

In the SC CO_2 method, the high-pressure cell was filled with a physical mixture of itraconazole-β-cyclodextrin. The system was then pressurized and heated up to the desired pressure and temperature and left in a static mode for 3 h. At the end of the process, the pressure in the cell was reduced to atmospheric

pressure within 15 min and the contents of the cell were ground and homogenized in a mortar.

2.3. Thermal analysis

Thermal analyses of the individual components or itraconazole-β-cyclodextrin combinations were performed using a differential scanning calorimeter (DSC 7, Perkin Elmer) with a nitrogen flow rate of 40 ml/min and a heating rate of 10 °C/min from 50 to 250 °C. Indium and zinc were used as standards.

2.4. UV spectroscopy

Quantitative analysis of the inclusion complexes were performed by UV spectrophotometry. Acetonitrile was used for the determination of free itraconazole content since β-cyclodextrin has poor solubility in acetonitrile and the inclusion complex is also expected to have low solubility in acetonitrile. To determine free itraconazole content, 10 mg of the sample was dissolved in 100 ml of acetonitrile. This solution was then sonicated for 5 min, filtered on a 0.45 μm filter (Millipore) and analyzed using a Philips PU 8620 UV spectrophotometer at 265 nm. The free itraconazole content was obtained from three measurements for each condition. Total itraconazole content in a 10 mg sample was determined from the initial drug to β-cyclodextrin molar ratio. The percentage of inclusion was evaluated by UV spectrometry, according to the following equation

$$\%\ \text{of inclusion} = \frac{\text{total itraconazole content} - \text{free itraconazole content}}{\text{total itraconazole content}} \times 100 \quad (1)$$

2.5. Dissolution rate

Dissolution profiles of itraconazole release were obtained using the paddle stirring method. Dissolution media consisted of enzyme-free simulated gastric fluid, which was prepared by dissolving NaCl (2 g) in 500 ml of deionized water and then concentrated HCl (7 ml) was added to adjust the pH to 1.4 ± 0.1. The final volume was adjusted to 1000 ml using deionized water. In all dissolution measurements 50 mg of drug or drug equivalent was dissolved in 500 ml of enzyme-free simulated gastric fluid (pH = 1.4 ± 0.1) at 37 ± 0.5 °C. The solution was mixed at 100 rpm for 60 min. One millilitre dissolution samples were collected at 5, 10, 20, 30, 45, and 60 min through 5 μm membrane filters. These samples were diluted to 10 ml with simulated gastric fluid. Samples of the diluted solutions were analyzed for itraconazole concentrations using a Philips PU 8620 UV spectrophotometer at 265 nm.

2.6. Animals

Male Wistar rats (180–200 g) were obtained from Faculty of Medicine and Health Sciences (FMHS) at UAE University after approval by the FMHS Animal Research Ethics Committee. These rats were maintained on a standard pellet diet and tap water *ad libitum*. The animals were kept in plastic cages (5 animals/cage) under a 12 h light/dark cycle and room temperature 22–24 °C. The rats were acclimatized to the environment for 2 weeks prior to experimental use. All experiments were conducted in compliance with rules and regulations of the FMHS Animal Research Ethics Committee for care and use of laboratory animals.

2.7. In vivo bioavailability

Itraconazole/β-CD products prepared by three different methods (physical mixing, coprecipitation, and supercritical CO_2 method) were given to the rats by ingestion using a gastric needle. In all experiments, itraconazole content was standardized (drug equivalent to 10 mg/kgb wt of the rat). Five rats were used for each drug preparation. Following light diethyl ether anesthesia, blood sample was collected from the retro-orbital plexus at 30-min intervals for 8 h [33]. Serum was collected and the proteins were precipitated (using 100 μl acetonitrile/100 μl serum), vortexed, centrifuged, and filtered (using 45 μm Millipore filter). The filtrate was dried using speed-vac and reconstituted in a phosphate buffer (K_3HPO_3, pH 7.8) solution and then injected into the HPLC as described previously [34] in order to determine the concentration of itraconazole in the blood.

In another set of experiments, three groups of five rats were studied to determine the deposition of itraconazole in liver and kidney. Each of the three groups was terminated at different time intervals (4, 6, and 8 h after ingestion) and liver and kidney tissues (1 g) were collected, freeze dried, powdered and homogenized in a phosphate buffer (K_3HPO_3, pH 7.8) solution. The homogenate was centrifuged ($400 \times g$ for 10 min). Proteins were precipitated using an equal volume of acetonitrile, then vortexed, centrifuged, and filtered using Millipore filter (45 μm). The filtrate was dried using speedvac and reconstituted in a phosphate buffer (K_3HPO_3, pH 7.8) solution and then injected into the HPLC for analysis.

2.8. Pharmacokinetic analysis

The area under the drug concentration–time curve from time 0 to 8 h ($AUC_{0-8\,h}$) was calculated by using Sigma Plot® 8.0, SPSS scientific graphing software (SPSS Inc., Chicago, IL, USA). The maximal plasma concentration of drug (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were directly obtained from plasma data.

2.9. Statistical analysis

SPSS (version 10) statistical program (SPSS Inc., Chicago, IL, USA) was used to carry out a one-way analysis of variance (ANOVA) on the obtained data. When significant differences by ANOVA were detected, analysis of differences between the means of different samples (prepared by supercritical CO_2, physical mixing and coprecipitation methods) were performed using Dunnett's *t*-test.

Table 1
Comparison between experimental inclusion data obtained from SC CO_2, physical mixture and coprecipitation methods

Temperature (°C)	Pressure (MPa)	Density (kg/m^3)	Inclusion yield (%)	
			1:2 mole–mole	1:4 mole–mole
50	25	834	1.60	1.61
	35	899	0.08	0.75
	45	944	0.04	2.25
100	25	588	4.65	21.37
	35	715	5.59	29.99
	45	790	7.70	31.44
130	25	471	6.40	31.17
	35	613	6.77	31.76
	45	703	8.28	33.53
Physical mixture			0.00	0.00
Coprecipitation			3.09	3.46

Itraconazole:β-CD (1:2 and 1:4 mole–mole ratio).

3. Results and discussion

3.1. Yield of inclusion complexes

Our previous results showed that β-cyclodextrin significantly improved solubility of itraconazole in aqueous solutions [29]. However, the maximum inclusion yield was 8.28%, which was obtained for the drug:CD (1:2 mole–mole) sample treated at a temperature and pressure of 130 °C and 45 MPa, respectively. In the current study the effect of SC CO_2 on the complex formation at different conditions (three levels of temperature: 50, 100, 130 °C; three levels of pressure: 25, 35, 45 MPa) was studied for a drug:CD ratio of 1:4. Since preliminary studies showed that increasing the exposure time ensures a better inclusion yield, 3 h was selected as the optimum time of exposure to SC CO_2. The effects of temperature, pressure, density of SC CO_2, and drug:CD ratio on inclusion yield are given in Table 1. Results of the SC CO_2 method are compared to those of physical mixture and coprecipitation methods for the two different itraconazole/β-cyclodextrin ratios (1:2 and 1:4). As Table 1 shows, significantly higher inclusion yields were obtained for the samples with a drug to cyclodextrin ratio of 1:4 as compared to those with 1:2 ratios. Moreover, the inclusion yield obtained by physical mixing (0% for both 1:2 and 1:4 drug:CD ratios) and coprecipitation methods (3.09% and 3.46% for 1:2 and 1:4 drug:CD ratio, respectively) were lower than those obtained by SC CO_2 method. For the products prepared by SC CO_2 method, the maximum inclusion yield was observed at the highest temperature (130 °C) and pressure (45 MPa) for both drug:CD ratios (8.28% for 1:2 and 33.53% for 1:4 ratio). This is attributed to the higher itraconazole solubility in SC CO_2 at the higher temperature and pressure [29].

Both temperature and pressure had significant effects on the inclusion yield (Table 1). Increasing temperature resulted in an increase in the inclusion yield at all pressures. However, increasing pressure increased inclusion yield only at the higher temperatures (100 and 130 °C). At 50 °C, inclusion yield was the highest at the lower pressure (25 MPa) for the sample with 1:2 drug:CD ratio, while at the same temperature the highest inclusion yield was obtained at 45 MPa for the sample with 1:4 ratio.

3.2. Analysis of inclusion complexes

DSC curves for pure itraconazole, pure β-CD as well as itraconazole/β-CD (1:2 and 1:4 mole–mole) products obtained by physical mixing, coprecipitation method and SC CO_2 method at three different temperatures (50, 100 and 130 °C), 3 h of static mode and three different pressures (25, 35 and 45 MPa), are shown in Fig. 1. These curves are normalized with respect to weight of itraconazole in the sample. Pure itraconazole showed a sharp melting endotherm at 165.2 °C. Pure β-CD showed two peaks: a broad endothermal effect, ranging between 50 and 150 °C corresponding to the dehydration of β-CD and a second peak at 219 °C characteristic of pure β-CD. DSC curve of the physical mixture for both drug:CD ratios (1:2 and 1:4) consisted of thermal profiles of drug and cyclodextrin with no significant change in the peaks, indicative of no drug–cyclodextrin interactions. This is consistent with the results of UV analysis (Table 1) indicating that no inclusion was formed in the physical mixtures. DSC curve for the product obtained by coprecipitation method showed a slight broadening of the endothermal peak for β-CD at 219 °C, indicative of some drug–cyclodextrin interaction. Results obtained by UV analysis (Table 1) also indicate that little inclusion (3.09% and 3.46% for the 1:2 and 1:4 ratios, respectively) was formed in the coprecipitation method. DSC curves of the itraconazole-β-CD samples treated with SC CO_2 show a peak at about 226 °C (7 °C higher than the endothermal peak of β-CD).

The endothermic peak corresponding to pure itraconazole, although reduced in size, was observed for the sample treated with SC CO_2, indicating an incomplete inclusion of the drug in the cyclodextrin cavity. This also agrees with the results of UV analysis (Table 1) showing an inclusion yield between 0.04% and 8.28% for the SC CO_2 products with 1:2 drug:CD molar ratio and an inclusion yield ranging from 0.75% to 33.53% for the 1:4 ratio. The endothermal peak corresponding to pure itraconazole was larger in the samples with 1:2 drug:CD ratio compared

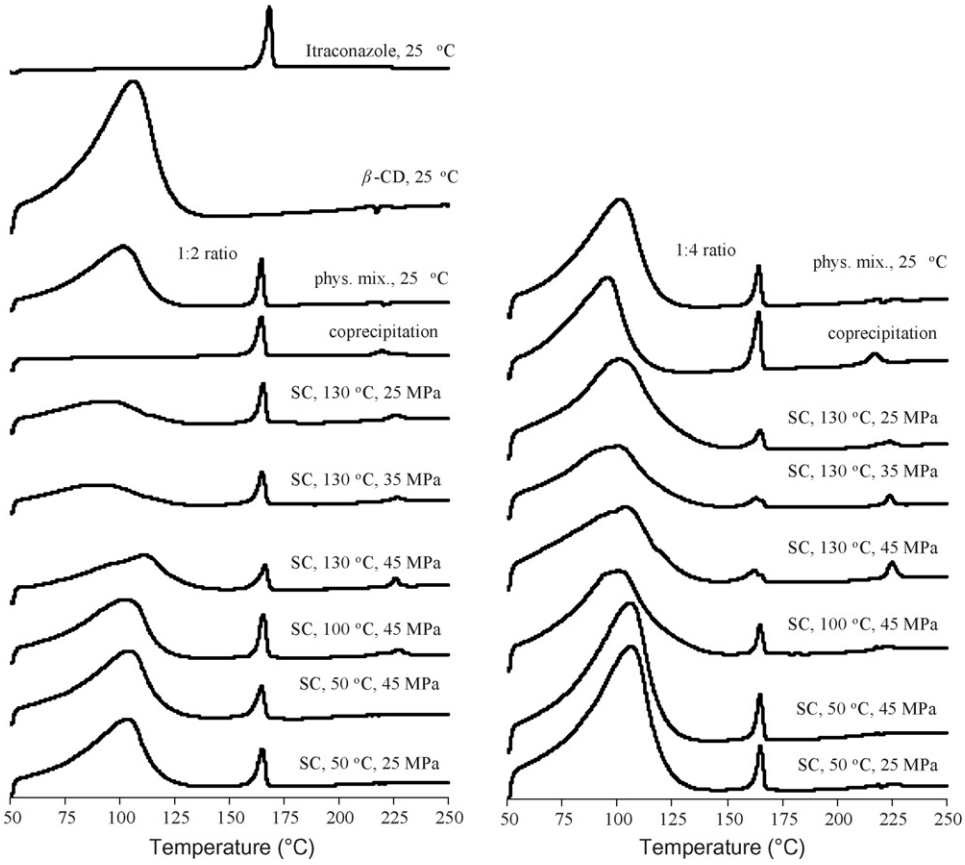

Fig. 1. DSC curves of pure itraconazole, pure β-CD, and itraconazole/β-CD (1:2 and 1:4 mole–mole) complexes prepared by physical mixing, coprecipitation method and SC CO_2 method at three different temperatures (50, 100 and 130 °C), 3 h of static mode and three different pressures (25, 35 and 45 MPa).

to that for the 1:4 ratio for most of the samples treated with SC CO_2 (especially at 100 and 130 °C). However, the peak at 226 °C was larger for the samples with 1:4 ratio as compared to that for the 1:2 ratio, suggesting higher inclusion yields for the products having a 1:4 drug:CD ratio, which is also confirmed by the UV analysis (Table 1). The product obtained at 130 °C and 45 MPa for the 1:4 drug:CD ratio resulted in the smallest endothermic peak corresponding to pure itraconazole and the largest peak at 226 °C among all samples studied. This suggests that the inclusion yield was highest for this product, as expected from the results of UV analysis (Table 1). These results indicate that higher amounts of cyclodextrin ensure higher degree of inclusion formation. Therefore, one can expect to obtain even higher inclusion yields than 33.53%, which was the maximum yield for the 1:4 drug:CD ratio if higher amounts of β-CD are used in the formulation. Comparing the peak size at 226 °C for the SC CO_2 products, it is clear that inclusion yield increased with temperature at all pressures while increasing pressure increased the inclusion yield only at the higher temperatures (100 and 130 °C), which is also in agreement with the inclusion yields obtained by UV analysis.

3.3. Dissolution studies

Dissolution profile of products obtained at different treatment pressures and temperatures was determined in order to see the effect of SC treatment condition on itraconazole dissolution in simulated gastric fluid. A typical dissolution curve is shown in Fig. 2, which presents the dissolution profiles of pure itraconazole, physical mixture of itraconazole/β-CD (1:2 mole–mole), and physical mixtures treated by supercritical CO_2

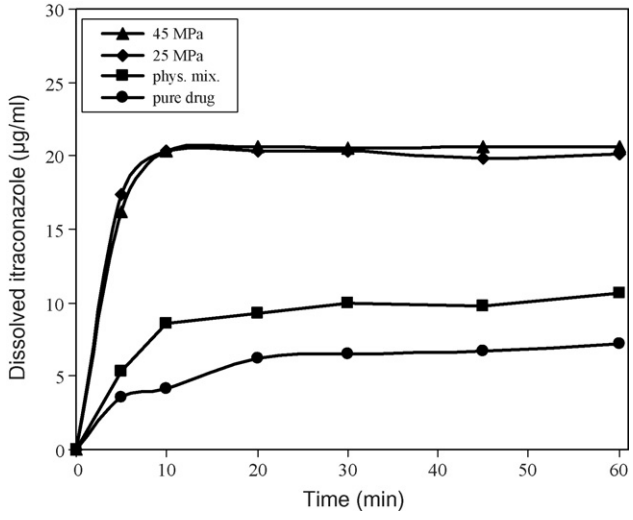

Fig. 2. Effect of treatment pressure on itraconazole dissolution profile. Treatment conditions: itraconazole/β-CD (1:2 mole–mole); $P = 25$ and 45 MPa; $T = 130$ °C; $t = 3$ h.

at two different treatment pressures (25 and 45 MPa) while keeping the temperature and treatment time constant at 130 °C and 3 h, respectively. Products obtained by SC CO_2 gave higher dissolution amounts than the pure itraconazole (by three times) and the physical mixture (by two times). This suggests that the inclusion yield was higher in the SC CO_2 products than in the physical mixture, which is consistent with results obtained from UV and DSC analysis. However, dissolution amounts were nearly the same for samples prepared by SC CO_2 at the two pressures, contrary to the fact that UV and DSC results indicated slightly higher inclusion yield for the sample prepared at 45 MPa. Moreover, samples prepared by the SC CO_2 method at lower treatment temperatures of 100 and 50 °C showed significantly lower itraconazole dissolution as compared to the sample prepared at 130 °C (figures for 100 and 50 °C are not shown here). The higher extents of dissolution obtained for the product prepared at 130 °C may be due to the higher inclusion yield for this sample as compared to the samples obtained at lower temperatures, in agreement with UV and DSC results.

3.4. Animal study

Serum concentrations of itraconazole after oral administration are shown in Fig. 3. Itraconazole was absorbed rapidly from gastrointestinal tract; the drug was detected in plasma after 30 min in all rats and reached its peak (T_{max}) at 120 min. The pharmacokinetic parameters of itraconazole, such as C_{max} and $AUC_{0–8\,h}$ were significantly different in the products prepared by the three different methods (Table 2). In SC CO_2 method, values of $AUC_{0–8\,h}$ were significantly higher than those observed in physical mixture and coprecipitation methods. Moreover, complexation of itraconazole with β-cyclodextrin using SC CO_2 (at 50 °C, 25 MPa, and 3 h) appears in the blood with the highest concentration (C_{max}) as compared to both physical mixture and coprecipitation methods. On the contrary the physical mixture of itraconazole with cyclodextrin showed the lowest dissolution rate and the lowest absorbed concentration due to the absence of inclusion complex in the physical mixture. Coprecipitation method also yielded lower absorption as compared to the complexation by SC CO_2 method. Drug clearance rate seemed to correlate with the serum blood concentrations. Two hours after ingestion, complexes prepared by SC CO_2 were much higher than the other two methods. Three hours later all drug complexes returned back to a nadir level. Itraconazole-β-cyclodextrin complexes prepared by SC CO_2 at other conditions will also be tested and are expected to show higher absorption than the sample treated with SC CO_2 at 50 °C, 25 MPa since analysis of inclusion complexes reveals higher inclusion yields at other conditions as discussed above.

Table 2
Pharmacokinetic data after oral administration of itraconazole/β-CD (1:2 mole–mole) products prepared by simple physical mixing, conventional coprecipitation method, and SC CO_2 method at 50 °C, 25 MPa, and 3 h of static mode

Preparation method	$AUC_{0–8\,h}$ (μg min/ml)	C_{max} (μg/ml)	T_{max} (min)
Physical mixture	5.75 ± 0.09*	1.30 ± 0.05*	120
Coprecipitation	8.51 ± 0.07*	2.93 ± 0.01*	120
SC CO_2	12.07 ± 0.17	4.54 ± 0.12	120

Statistical analysis was performed by Dunnett's *t*-test after ANOVA analysis.
* $P < 0.001$ vs. SC CO_2 group. Data are expressed as mean ± S.E.M. ($n = 5$).

Itraconazole concentrations in both liver (Fig. 4) and kidney tissues (Fig. 5) showed that drug appears in both tissues with the highest itraconazole concentration in all animals after 4 h and sustains up to 6 h. Thereafter, the drug was eliminated from the body. These data document that the complexation of itraconazole with β-cyclodextrin using SC CO_2 (at 50 °C, 25 MPa) increased drug solubility as compared to the other two methods. Liver and kidney tissues were collected after 2 h and itraconazole was not detected (data are not shown). Results show that complexation of itraconazole with β-cyclodextrin using SC CO_2 was increased ($P < 0.01$) as compared to complexation with physical mixing or coprecipitation methods. Drug–cyclodextrin complexes pre-

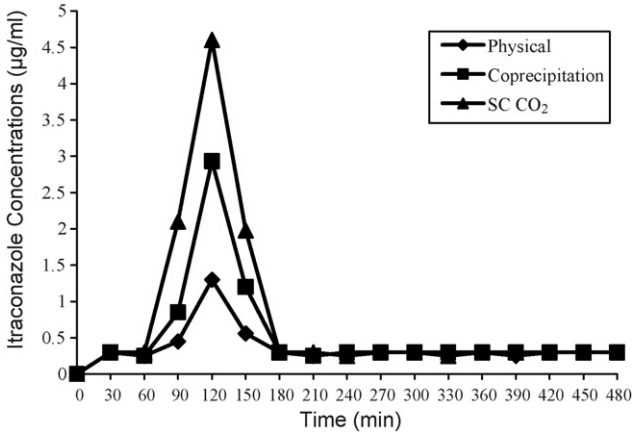

Fig. 3. Plasma concentration–time profile after oral administration of itraconazole/β-CD (1:2 mole–mole) products prepared by simple physical mixing, conventional coprecipitation method, and SC CO_2 method at 50 °C, 25 MPa, and 3 h of static mode. Data are expressed as mean ± S.E.M. ($n = 5$).

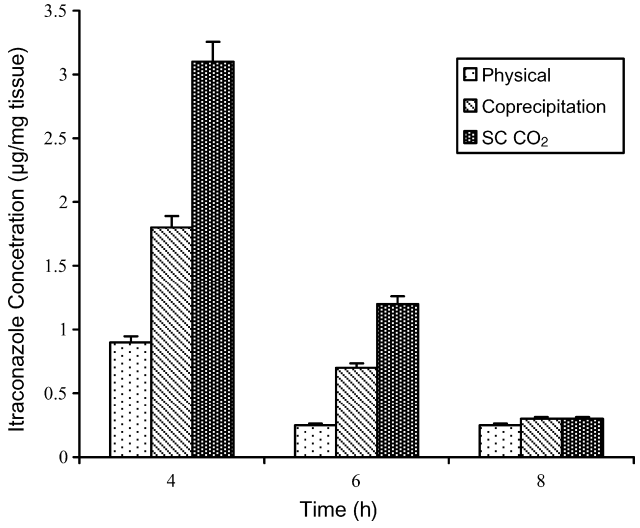

Fig. 4. Itraconazole concentration in liver tissue after ingestion of itraconazole/β-CD (1:2 mole–mole) products prepared by simple physical mixing, conventional coprecipitation method, and SC CO_2 method at 50 °C, 25 MPa, and 3 h of static mode.

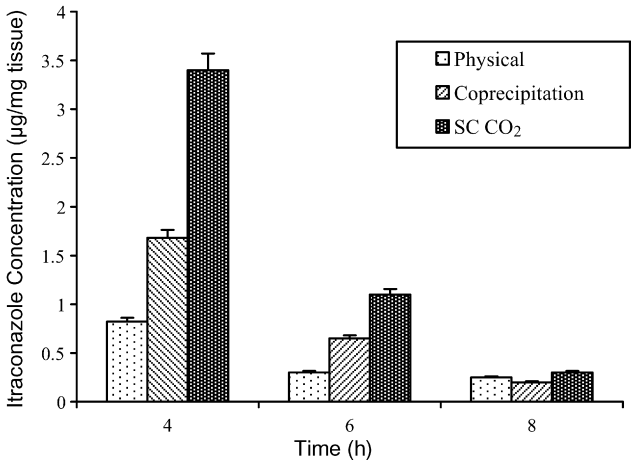

Fig. 5. Itraconazole concentration in kidney tissue after ingestion of itraconazole/β-CD (1:2 mole–mole) products prepared by simple physical mixing, conventional coprecipitation method, and SC CO_2 method at 50 °C, 25 MPa, and 3 h of static mode.

pared by SC CO_2 at other conditions will also be studied in liver and kidney tissues in an attempt to obtain higher *in vivo* bioavailabilities.

4. Conclusions

Supercritical CO_2 proved to be a novel and dependable method for preparing inclusion complexes between itraconazole and β-CD. The formation of solvent-free inclusion complexes prepared using SC CO_2 was verified by UV and DSC analysis and compared to those obtained by physical mixing and coprecipitation methods. Thermal analysis of the products obtained by different methods were consistent with the results of UV analysis indicating no inclusion formation in the physical mixture, small inclusion yield for the coprecipitated product and relatively high inclusion formation for the product obtained by SC CO_2.

Results of the dissolution studies were also consistent with the results obtained from UV and DSC analysis, showing higher dissolution amounts for the product obtained by SC CO_2 as compared to the pure itraconazole and the physical mixture. Moreover, dissolution results were not affected by the pressure as much as by the temperature of SC CO_2, suggesting that treatment temperature may be more important than treatment pressure in the formation of inclusion complex between itraconazole and β-CD. However, UV and DSC results showed that both temperature and pressure had significant effects on the interaction between itraconazole and β-CD and consequently on the formation of inclusion complexes. More experiments are currently underway to explain this discrepancy.

In vivo drug pharmacokinetic studies showed that ingestion of itraconazole complexed with β-cyclodextrin using the solvent-free SC CO_2 results in a higher bioavailability of itraconazole as compared to the products obtained by physical mixing or coprecipitation method, indicating the superiority of the SC CO_2 method as compared to the physical mixing or coprecipitation methods. Moreover, itraconazole-β-cyclodextrin complexes prepared using SC CO_2 made itraconazole available to liver and kidney tissues for at least 6 h after ingestion.

Itraconazole-β-CD complexes prepared by SC CO_2 should provide minimal side effects (related to the use of organic solvents) in human body since this method has no toxic solvent residuals. Efficacy, safety and pharmacokinetics of binary inclusion complexes obtained by SC CO_2 at other conditions will also be investigated in both *in vitro* and *in vivo*. The SC CO_2 method is also being tested with other drug–CD systems. Inclusion experiments in which drug and cyclodextrin are loaded in separate cells will also be investigated.

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