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Preparation and evaluation of itraconazole dihydrochloride for the solubility and dissolution rate enhancement

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ABSTRACT

The purpose of this work was to explore the feasibility of preparing itraconazole hydrochloride to improve the solubility and dissolution rate. Itraconazole dihydrochloride was synthesized by bubbling anhydrous hydrogen chloride gas into the acetone suspension of itraconazole. Results of the elementary analysis gave the molecular formula of $C_{35}H_{38}Cl_2N_8O_4{\cdot}2HCl$ and its structure was confirmed by Fourier transform infrared (FTIR), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Powder X-Ray diffraction (PXRD) suggested that a new crystalline form of the salt was formed. The morphology and mean size distribution study by scanning electron microscopy (SEM) and dynamic light scattering (DLS) confirmed that the salt was dispersable nanoparticle aggregation. Aqueous solubility measurements showed that the solubility of the salt, its 1:1, 1:2 and 1:3 (w/w) physical mixtures with beta-cyclodextrin (β-CD) was 6, 99, 236 and 388 times greater than itraconazole. More than 94% of itraconazole was dissolved out of the salt/β-CD 1/3 physical mixture after 60 min. The stability studies indicated that the physical mixture remained stable for 24 months in assay, the related substances and dissolution. Based on the present results, it is concluded that hydrochloride formation can significantly increase solubility and dissolution rate of itraconazole, and the formulation of itraconazole dihydrochloride/β-CD (1/3) would be an environment-friendly, economic and practical alternative to the commercially available itraconazole capsules (Sporanox®).

1. Introduction

Itraconazole ($C_{35}H_{38}Cl_2N_8O_4$) is an active triazole antifungal agent. Because of the highly hydrophobic characteristics and extremely weak basicity, its aqueous solubility is estimated at approximately 1 ng/ml at neutral pH and approximately 4 μg/ml at pH 1. The pK_{a4} is determined to be 4, pK_{a3} is calculated to be 1.5–2, while its other ionizable nitrogens (pK_{a1} and pK_{a2}) are not protonated between pH 2 and pH 10 (Peeters et al., 2002). In the formulation of commercially available itraconazole capsules (Sporanox®), the solid dispersions of the drug and hydroxypropylmethylcellulose (HPMC) were developed to enhance its solubility and dissolution (Gilis et al., 1997). The toxic solvent dichloromethane was used to form a uniform drug/HPMC solution. This solution was sprayed on the sugar beads, dried to remove the organic solvents. Polyethylene glycol (PEG)20000 in dichloromethane and ethanol was sprayed on the drug-coated sugar beads to prevent sticking of the beads. The twice-coated beads were filled into hard-gelatin capsules. The above manufacturing process of Sporanox® included several drying steps for long periods of time in order to remove of hazardous dichloromethane.

In order to avoid the organic solvents, melt extrusion technology offers a promising alternative (Verreck et al., 2003; Six et al., 2003,2005). Solid dispersions made up of itraconazole and HPMC (40:60, w/w) were manufactured by hot-melt extrusion and filled in gelatin capsules. However, high temperature, long residence time and high shear force might result in drug and matrix degradation.

Supercritical carbon dioxide (SC CO_2) (Hassan et al., 2004) was reported to replace toxic solvents. In the study, SC CO_2 was added to following formulation ingredients: itraconazole, PEG20000, HPMC, sodium starch glycolate and glycerol (1:2.75:0.25:0.25:0.75) in order to form a uniform itraconazole solution, which is highly porous when solidified. Itraconazole dissolution of 100% at 1-h was achieved. But the process needed high temperature (135 °C) and pressure (300 atm) for 30 min, which may not be a practical method for large scale manufacture.

Besides the solid dispersions, the inclusion complex of itraconazole with beta-cyclodextrin (β-CD) using SC CO_2 was reported (Al-Marzouqi et al., 2006; Hassan et al., 2007). At a temperature of 130 °C and pressure of 45 MPa, the maximum inclusion yield

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was only 8.28%, although the molecular ratio of drug: β-CD was 1:2. However, the enhancement of *in vitro* dissolution and *in vivo* bioavailability was not significant.

Salt formation is often used to increase drug solubility in parenteral and other liquid formulations. It is also the most common approach of increasing solubility, dissolution rate of poorly water-soluble drugs in solid dosage forms. Of approximately 300 new chemical entities approved by the FDA during the 12 years from 1995 to 2006 for marketing, 120 were in salt forms. In addition, out of the 101 approved salts of basic drugs, 54 salts were prepared with hydrochloric acid, indicating the hydrochloride was the predominant salt form (Serajuddin, 2007).

The purpose of present work was to explore the feasibility of the hydrochloride salt to improve the solubility and dissolution rate of itraconazole. Itraconazole dihydrochloride was prepared by bubbling anhydrous hydrogen chloride gas into the acetone suspension of itraconazole, and characterized by scanning electron microscopy (SEM), powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC). Physical mixtures containing different ratios of itraconazole dihydrochloride and β-CD were prepared. The formulations were evaluated for their solubility, *in vitro* dissolution and stability.

2. Materials and methods

2.1. Materials

Itraconazole (purity more than 99.5%) was provided by department of medicinal chemistry, Shanghai Institute of Pharmaceutical Industry. Commercially available itraconazole capsules (Sporanox®, 100 mg/capsule) were purchased from Xian Janssen Pharmaceutical Ltd. (Xian, China). β-CD was purchased from Guangdong Yunan Cyclodextrin Ltd. (Guangdong, China). All other chemicals were of analytical grade.

2.2. Preparation of itraconazole dihydrochloride

Itraconazole (40 g) was suspended in 800 ml of acetone, and into the suspension heated under reflux, the anhydrous gas of hydrogen chloride was bubbled slowly. After about 30 min, the suspension became a solution and in another 5–10 min, the precipitate of the salt was formed. The pass of hydrogen chloride lasted for 2 h and the mixture was allowed to stand overnight at room temperature. The product was collected by filtration, washed with acetone and dried at 105 °C.

2.3. Fourier transform infrared (FTIR) spectroscopy

FTIR absorption spectra of itraconazole and itraconazole dihydrochloride were recorded using a spectrometer (Nexus 670, Nicolet Instrument Co.) equipped with a DTGS detector. KCl disks were prepared (2 mg sample in 200 mg KCl) and scanned over a range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.4. Thermogravimetric analysis (TGA)

The weight loss of itraconazole dihydrochloride upon heating was determined by thermogravimetric analyzer (TGA 7, PerkinElmer Inc.) with nitrogen flow rate of 40 ml/min and a heating rate of 10 °C/min from 50 to 300 °C.

2.5. Differential scanning calorimetry (DSC)

Thermal behavior of itraconazole and itraconazole dihydrochloride were recorded using a differential scanning calorimeter (DSC 7, PerkinElmer Inc.) with nitrogen flow rate of 40 ml/min and a heating rate of 10 °C/min from 25 to 400 °C.

2.6. Powder X-Ray diffraction (PXRD)

The PXRD patterns of itraconazole and itraconazole dihydrochloride were measured by the X-ray diffractometer (D/max-rB, Rigaku), with CuKα radiation, voltage 40 KV, current 60 mA, scan range 3–50°, scan rate 4°/min.

2.7. Scanning electron microscopy (SEM)

SEM photomicrographs were taken to compare the crystal morphology of itraconazole and itraconazole dihydrochloride. SEM micrographs were taken using scanning electron microscopy (XL30, Philips Analytical Inc.). Samples were coated with gold before examination.

2.8. Dynamic light scattering (DLS)

The mean diameter and diameter distribution of itraconazole dihydrochloride were determined by dynamic light scattering in Nicomp™ 380 ZLS particle sizer (Particle Sizing Systems Inc., Santa Barbara, CA, USA). 10 mg of itraconazole dihydrochloride was suspended in 10 ml of acetonitrile. The sample suspension was sonicated for 10 min, and determined promptly.

2.9. Preparation of physical mixtures

Physical mixtures of itraconazole dihydrochloride with β-CD were prepared by passing the raw drug with β-CD (1:1, 1:2, 1:3 (w/w), respectively) through a 60-mesh sieve repeatedly until homogeneous mixtures were obtained. The physical mixture of itraconazole with β-CD (1:3) was prepared by the same method.

2.10. UV spectroscopy

Quantitative analysis of itraconazole in solubility and dissolution testing were performed by UV spectrophotometry at 254 nm. The method validation results showed that β-CD did not interfere with the spectrophotometric determination.

2.11. High performance liquid chromatography (HPLC)

The assay and related substances of itraconazole dihydrochloride and its capsules were determined by reverse phase HPLC (Waters, USA) in Supelco C18 column (5 μm, 25 cm × 4.6 mm ID). The mobile phase consisting of methanol/0.5% ammonium acetate (80:20, v/v) was filtered through a membrane filter (0.22 μm), and degassed by ultrasonication before use. The flow rate was 1.5 ml/min, and the UV detection wavelength was 260 nm.

2.12. Solubility measurement

Excess amounts of itraconazole and itraconazole dihydrochloride were added to 8 ml of simulated gastric fluid (pH 1.2 ± 0.02). The suspensions were sonicated for 15 min and were shaken at constant temperature (37 ± 0.5 °C). After 72 h, the suspensions were filtered through a disposable syringe filter (0.45 μm) and appropriately diluted with simulated gastric fluid. The amount of dissolved itraconazole was quantified by UV spectroscopy.

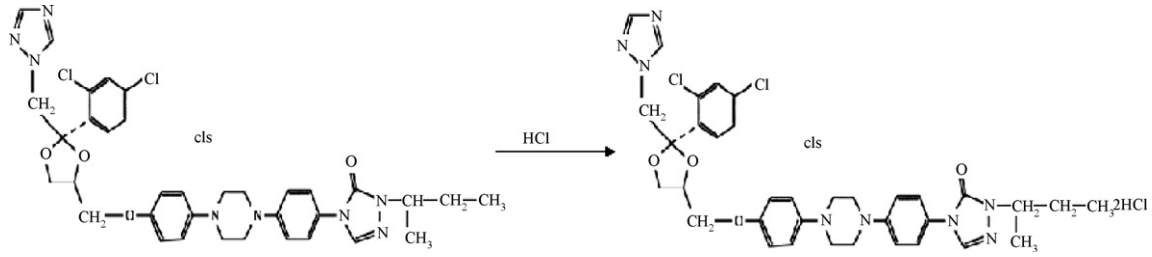

Fig. 1. The schematic presentation of itraconazole dihydrochloride formation.

2.13. Dissolution testing

Dissolution profiles of itraconazole, itraconazole dihydrochloride/β-CD physical mixtures and Sporanox® were determined in simulated gastric fluid. Dissolution testing was conducted in 37 ± 1 °C, 900 ml USP VII enzyme-free simulated gastric fluid (pH 1.2 ± 0.02). The accurately weighed samples equivalent 100 mg of itraconazole were filled in size 0 gelatin capsules. The capsule was placed in basket sinker of USP dissolution apparatus. The dissolution media was stirred at 100 rpm in the paddle method. 5 ml dissolution samples were collected at 5, 10, 20, 30, 45, 60 and 80 min, respectively, through 0.45 μm membrane filters, meanwhile an equal volume of fresh dissolution media maintained at the same temperature was added after withdrawing sample to keep the volume of dissolution media constant. 2 ml of filtered samples were diluted to 10 ml with simulated gastric fluid. Samples of the diluted solutions were analyzed for itraconazole concentrations by UV spectroscopy.

2.14. Stability testing

Stability testing was conducted under the storage condition of 25 ± 2 °C/65 ± 5% RH. Itraconazole dihydrochloride and its capsules filled with the physical mixture (salt: β-CD 1:3, w/w) were packaged in closed aluminum-polyethylene laminated bags. Assay and the related substances were evaluated by HPLC. Dissolution was assessed as described above. Assay, related substances and dissolution were tested on 0, 3, 6, 9, 12, 18 and 24 months.

3. Results

3.1. Salt formation

42 g of white powder was obtained with a yield of 95%. Elementary analysis (%, found/calculated based on $C_{35}H_{38}Cl_2N_8O_4 \cdot 2HCl$, Fig. 1) C: 53.70/53.99; H: 5.20/5.18; N: 14.46/14.39; Cl: 18.20/18.22.

3.2. FTIR spectra

Fig. 2 showed the FTIR spectra of itraconazole and itraconazole dihydrochloride. The characteristic absorption peak of hydrochloride salt was observed at 2161 cm^{-1}. And 3403 cm^{-1} might be attributed to O–H bond stretching vibration, implying a small amount of adsorbed water in the sample. The other characteristic absorptions in the FTIR spectra of itraconazole dihydrochloride were similar to those of itraconazole.

3.3. TGA plot

TGA data of itraconazole dihydrochloride showed the weight loss of 0.391% in the temperature interval 60–80 °C, and 9.581% in the temperature interval 108–255 °C. The former might result from a small amount of adsorbed water in the sample, the latter implied the complete dehydrochlorination of the salt, since the hydrochloride acid contributed 9.37% by weight in itraconazole dihydrochloride, almost equal to the weight loss between 108 and 225 °C.

3.4. DSC curves

Fig. 3 showed the DSC curves of itraconazole and itraconazole dihydrochloride. Itraconazole was characterized by a single, sharp melting endotherm at 171 °C, in agreement with its melting point (mp). However, the salt did not show any characteristic endotherm peak. This was confirmed by mp determination of itraconazole dihydrochloride, in which no sharp mp was observed.

3.5. PXRD patterns

Fig. 4 showed PXRD patterns of itraconazole and itraconazole dihydrochloride. The new sharp peaks, significantly differing from the original base in position and intensity, was observed in the PXRD patterns of the salt, which was contributed to a new crystalline form of the salt.

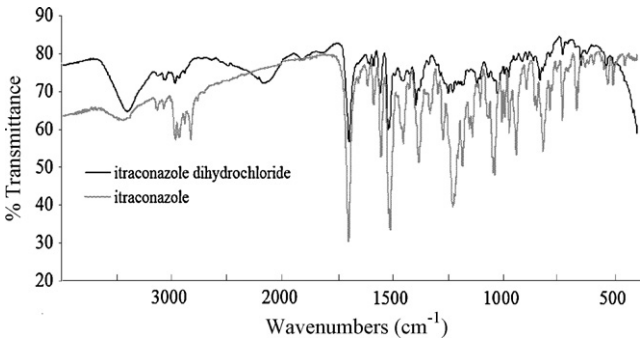

Fig. 2. FTIR spectra of itraconazole and itraconazole dihydrochloride.

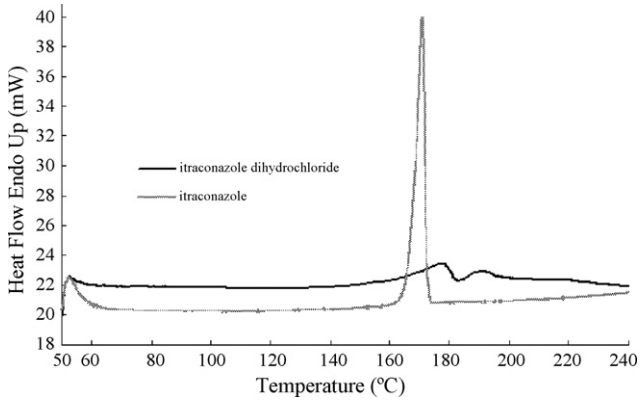

Fig. 3. DSC curves of itraconazole and itraconazole dihydrochloride.

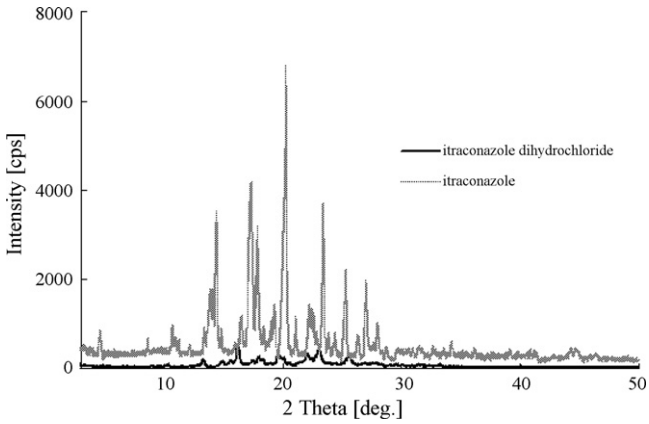

Fig. 4. PXRD patterns of itraconazole and itraconazole dihydrochloride.

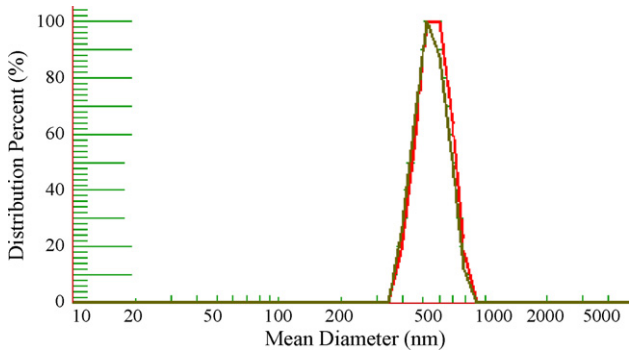

Fig. 6. Diameter distribution profile of itraconazole dihydrochloride.

3.6. SEM study

The SEM photomicrograph of itraconazole revealed solid cubes (Fig. 5a), which was similar to Al-Marzouqi's reports (Al-Marzouqi et al., 2006). In contrast, the morphology of itraconazole dihydrochloride was the aggregated nanoparticles (Fig. 5b). To investigate the nano behavior, the bulk salt was dispersed in acetonitrile as described above, the dispersoid was then dried in a vacuum, coated with gold and measured (Fig. 5c). The high magnification SEM morphology revealed dispersed salt particles with a geometric diameter of <1000 nm.

3.7. DLS study

Itraconazole dihydrochloride had a sharp diameter distribution around its average diameter of 546 nm (Fig. 6). The DLS result confirmed the nanostructure of itraconazole dihydrochloride powder observed by SEM study.

Table 1
Effects of hydrochloride formation and physical mixing with β-CD on solubility of itraconazole in 37 °C simulated gastric fluid.

	Solubility[a] in 37 °C simulated gastric fluid (μg/ml)
Itraconazole	11.58 ± 0.07
Itraconazole/β-CD 1/3 (w/w) physical mixture	720.73 ± 33.20
Itraconazole dihydrochloride (Itra·2HCl)	73.51 ± 6.94
Itra·2HCl/β-CD 1/1(w/w) physical mixture	1142.80 ± 32.03
Itra·2HCl/β-CD 1/2 (w/w) physical mixture	2729.45 ± 158.00
Itra·2HCl/β-CD 1/3 (w/w) physical mixture	4490.83 ± 63.00

[a] Each solubility data represents mean ± S.D. (n = 3).

3.8. Solubility study

Table 1 illustrated that hydrochloride formation significantly improves the solubility of itraconazole in simulated gastric fluid. In 37 °C simulated gastric fluid, the solubility of itraconazole was about 11 μg/ml. Meanwhile itraconazole dihydrochloride was six times more soluble than its base.

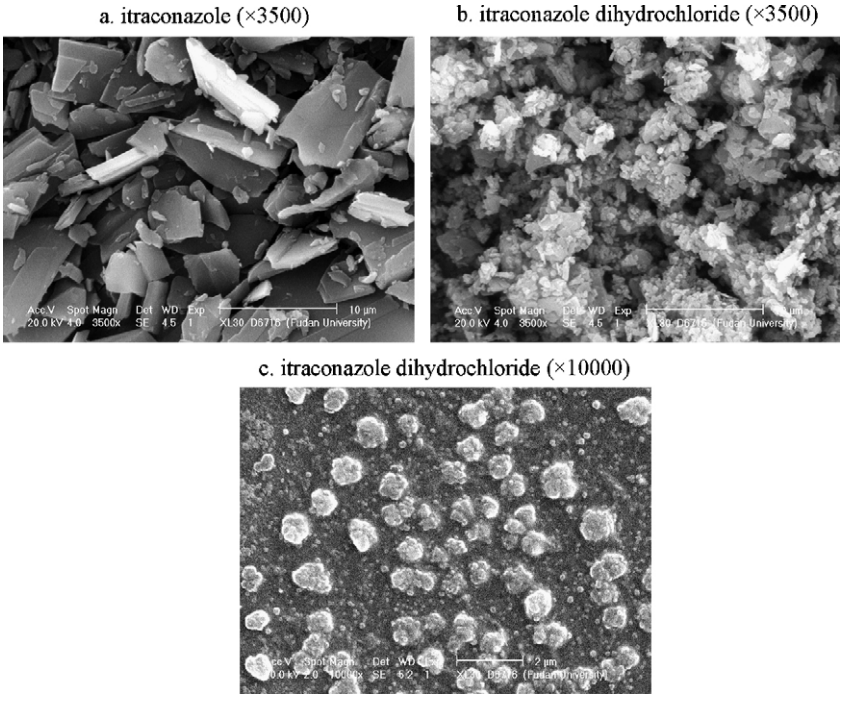

Fig. 5. SEM micrographs of itraconazole and itraconazole dihydrochloride.

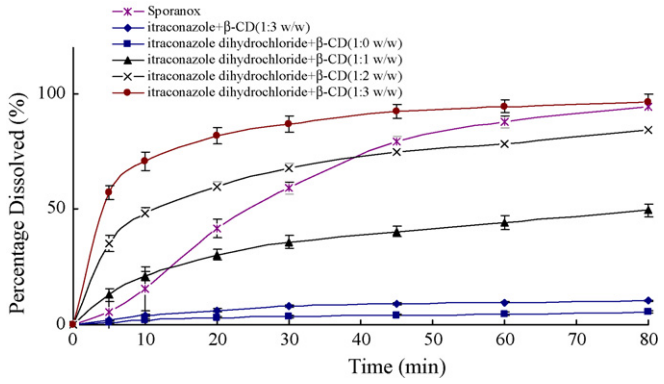

Fig. 7. Dissolution profiles of itraconazole/β-CD physical mixture, itraconazole dihydrochloride/β-CD physical mixtures and Sporanox® in 900 ml of simulated gastric fluid (pH 1.2) and a stirring of 100 rpm. Results were expressed as mean ± S.D. (n = 6).

In the solubility study of itraconazole dihydrochloride, viscous bulk drug precipitates were observed, indicating that salt nanoparticle structure was destroyed in simulated gastric fluid. To stabilize the nanoparticles against aggregation and agglomeration, β-CD was selected as a disperser. It might function as a solubilizer, too, since cyclodextrins was reported to solubilize itraconazole by inclusion (Peeters et al., 2002; Al-Marzouqi et al., 2006). As seen from Table 1, the solubility increased with increasing β-CD concentration in drug/β-CD physical mixtures. The maximum solubility was 4491 μg/ml when itraconazole dihydrochloride was mixed with β-CD (1:3, w/w).

3.9. Dissolution testing

Fig. 7 presented the dissolution profiles of itraconazole/β-CD physical mixture, itraconazole dihydrochloride/β-CD physical mixtures and Sporanox®. Less than 10% of itraconazole was dissolved out of itraconazole/β-CD 1/3 (w/w) physical mixture after 60 min, showing that dispersing and solubilizing effects of β-CD was not enough to provide an efficient dissolution. After hydrochloride formation, the dissolution rate increased as a function of β-CD content in physical mixtures, the rank of dissolution rate of the four testing formula was as follows: 1/3 > 1/2 > 1/1 > 1/0. More than 94% of itraconazole was dissolved from itraconazole dihydrochloride/β-CD 1/3 (w/w) physical mixture after 60 min, while only 87.8% of itraconazole was dissolved for Sporanox® under the same condition.

Table 2
Pharmacokinetic parameters (mean ± S.D.) of itraconazole after the randomized cross-over administration of Sporanox® and itraconazole dihydrochloride capsules in 20 healthy volunteers.

Parameters	Sporanox®	Itraconazole dihydrochloride capsules
t_{max} (h)	4.2 ± 0.70	3.9 ± 0.70
C_{max} (μg/l)	77.8 ± 45.2	81.4 ± 60.0
$t_{1/2}$ (h)	29.3 ± 5.81	29.3 ± 5.62
$AUC_{0-72\,h}$ (μg h/l)	1174.3 ± 701.9	1199.4 ± 649.6
$AUC_{0-\infty}$ (μg h/l)	1386.1 ± 735.8	1414.0 ± 815.2

3.10. Stability study

No significant change of assay, related substances and dissolution occurred during 24 months' testing at the long-term storage condition. The results indicated that itraconazole dihydrochloride and its physical mixture with β-CD (salt: β-CD 1:3, w/w) were chemically and physically stable.

4. Discussion

Clinical study of itraconazole dihydrochloride/β-CD physical mixture (salt: β-CD 1:3, w/w) capsules was performed (Miao et al., 2006). A randomized, crossover study of 20 healthy volunteers receiving single oral dose of 200 mg itraconazole was conducted with *in vivo* blood concentrations determined by HPLC-fluorescence detection. The pharmacokinetic parameters for the testing itraconazole dihydrochloride capsules and the reference Sporanox® were listed in Table 2. There were no significant differences in main pharmacokinetics parameters between the two preparations, except in t_{max} from analysis of variance and one-side and two-sides t-tests. The relative bioavailability of the testing itraconazole dihydrochloride capsule was 105.3 ± 23.4%. The results demonstrated that formation of dihydrochloride salt could effectively improve the *in vivo* absorption and bioavailability of itraconazole.Recently, nanotechnology is emerging as an effective tool for resolving challenge in delivering poorly water soluble drugs.

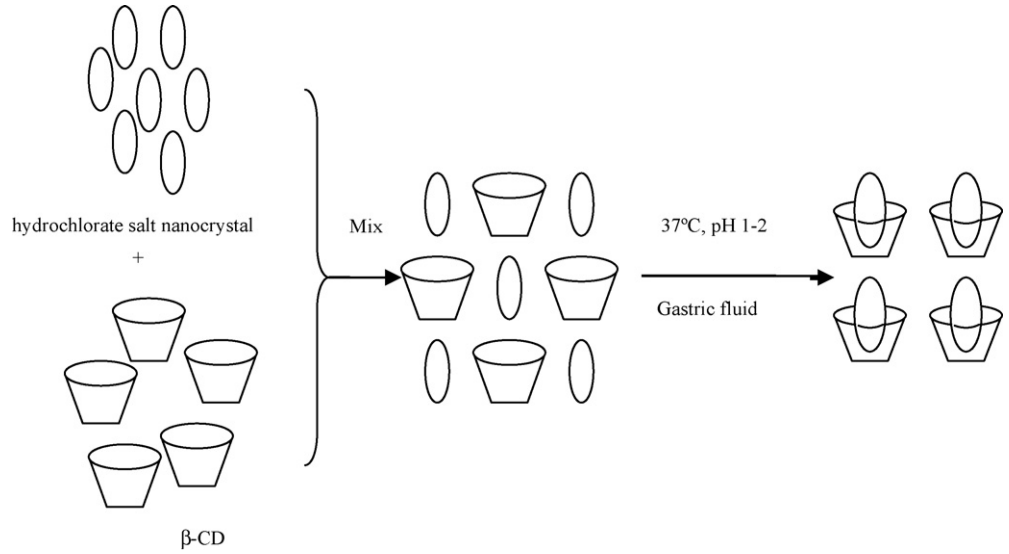

Fig. 8. The schematic process of inclusion complex formation of itraconazole dihydrochloride and β-CD.

NanoCrystal technology can improve the bioavailability of drugs by milling drug particles using a wet-milling technique to less than 1000 nm, and stabilizing the nanoparticles against agglomeration using surface adsorption of suitable stabilizers (Arnum, 2007).This study implies that salt formation may be a versatile approach of preparing nanocrystals of poorly water soluble itraconazole, and cyclodextrins will be a valuable stabilizer of nanocrystals because of their well-known solubilizing effects. The profound improvements in the solubility and dissolution of itraconazole may be attributed (1) to the nanocrystal nature of itraconazole dihydrochloride salt: reduced particle size and increased surface area, (2) to the dispersing effects of β-CD on the hydrochloride salt, preventing the salt nanocrystal of aggregation and agglomeration and (3) to the inclusion effects of β-CD on itraconazole dihydrochloride (Fig. 8).

5. Conclusion

Based on the present results, it is concluded that formation of hydrochloride salt could significantly improve the solubility and dissolution rate of itraconazole, and the salt/β-CD 1/3 (w/w) physical mixture formulation would be an environment-friendly, economic and practical alternative to Sporanox®.

Acknowledgments

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