



Preparation and Characterization of a Novel Preparation of Itraconazole Nanoparticles with Improved Dissolution and Bioavailability

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ABSTRACT

The aim of the present work is to utilize the nanotechnology for preparing stable nanoparticles that enhance the dissolution and hence the bioavailability of poorly water-soluble Itraconazole (ITZ). ITZ nanosuspensions were produced by nanoprecipitation method in the presence of surfactant (tween 80) and selected stabilizers (HPMC, PVP K 17 and Pluronic F 68) at different ratios with the drug. The nanosuspensions were evaluated for their physicochemical properties and drug content. The nanoparticles were prepared by lyophilization technique and characterized by morphology evaluation, Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC) and X-ray diffraction (XRD). The optimized formulation showed an average particles size of 213.56 ± 19.58 nm and Zeta potential of -18.35 ± 2.55 mV. *In vitro* cumulative release from the nanosuspension was 85.64% at 60 min when compared to pure drug 35.52% and freeze dried nanoparticles 93.81%. Pharmacokinetic studies revealed that $AUC_{(0-36)}$ was increased by two folds when ITZ nanoparticles were administered orally compared with the market formulation (Sporanox[®]), which in turn increased the bioavailability. Thus, Nanoparticles seems to be a promising approach for increasing the dissolution and enhancement the bioavailability of ITZ.

Key words: Itraconazole, Nanoprecipitation method, Stabilizer type, Nanosuspension, Bioavailability Study.

INTRODUCTION

Nanosuspension technology is applicable to all poorly soluble drugs and an outstanding feature of this technology is its simplicity. Nanosuspensions can be applied to various administration routes such as oral, parenteral, pulmonary, ophthalmic, and nasal routes. Administration of poorly water-soluble drug in the form of nanosuspensions has shown increased onset of action, dissolution rate and enhanced the bioavailability [1, 2]. A pharmaceutical nanosuspension is defined as very finely, biphasic, dispersed solid drug particles in an aqueous vehicle, size below 1 μ m stabilized by surfactants and polymers that prepared by suitable methods for drug delivery applications [3, 4]. ITZ is an orally active triazole antimycotic agent, which is active against a broad spectrum of fungal species including *Cryptococcus*, *Candida*, *Aspergillus*, *Blastomyces* and *Histoplasma capsulatum* var *capsulatum* [5, 6]. ITZ is a weak basic drug ($pK_a = 3.7$), that almost ionized at only low pH [7, 8] has extremely low water solubility (about 1 ng/ml at neutral pH). The log partition coefficient of ITZ in a system of n-octanol with an aqueous buffer solution of pH 8.1 is 5.66, representing a very high

lipophilicity [9,10]. The efficiency of azole antifungal drugs is low because of poor water solubility [11]. Nanoprecipitation method presents numerous advantages, in that it is a straight forward technique, rapid and easy to perform. In this method, the drug is dissolved in an organic solvent such as acetone, acetonitrile, methanol or ethyl acetate. The organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentration of stabilizer and homogenizer speed. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed. The supersaturation is further accentuated by evaporation of drug solvent, this yields to the precipitation of the drug. High shear force prevents nucleus growth and Oswald's ripening [12].

In the present study, nanoprecipitation technique is used where the drug solution in a water miscible organic solvent which is mixed with an aqueous solution containing a surfactant and different types of stabilizers. Upon mixing, the supersaturated solution leads to nucleation and growth of drug nanoparticles, which may be stabilized by

surfactants. The aim of this work is to optimize and characterize the formulations prepared by nanoprecipitation method for the preparation of ITZ nanoparticles

MATERIALS AND METHODS

Materials: Itraconazole was kindly supplied by Adwia Pharma, Egypt. Hydroxypropyl methylcellulose K15M (HPMC) was kindly supplied by EPICO Pharmaceutical Company, Egypt. Inutec SP1® was kindly supplied by Beneo Biobased Chemicals, Belgium. Pluronic F68 by Sigma-Aldrich, Inc., Germany. Tween 80, ethyl alcohol 95% V/V by from EL-Nasr pharmaceutical chemical CO. (Egypt). Every additional chemical or reagent was of analytical grade or better.

Methods

Preparation of ITZ Nanosuspensions:

Nanosuspensions were developed according to the nanoprecipitation method [13-15]. In brief, ITZ (10 mg) was dissolved in 2 ml ethanol (95% V/V) at room temperature (25±1°C) to form uniform organic solution. The aqueous solution containing surfactant and selected stabilizers (HPMC, PVP K 17 and Pluronic 68) at different ratios (4:1), (2:1) and (1:1) drug to stabilizers (Table 1). The surfactant (tween 80) and selected stabilizer were dissolved in 50 ml deionized water to form antisolvent system. This was followed by adding the organic solution into stabilizer/surfactant aqueous solution at very slow rate (0.5ml/min) by the help of syringe, under high speed mechanical agitation of 8000 rpm using Probe sonicator (model VC 505) for 30 minutes to get desired nanosuspension. Low temperature (below 8 °C) was maintained throughout the process using an ice-water bath which controlled the precipitation rate. The different formulations were prepared according to the design in Table 1. The suspensions were kept under vacuum at room temperature for 2 hours to remove organic solvents. The suspensions formed were kept in a 4 °C till further use.

Characterization of the Prepared ITZ Nanosuspensions

Particle size analysis: Particle size and polydispersity index (PI) of the prepared ITZ nanosuspensions were measured by Photon Correlation Spectroscopy (PCS) using a Zetasizer Nano ZS-90 instrument. In order to analyse, an aliquot of the nanosuspension was diluted before the measurement. Measurements were performed in triplicate at 90° scattering angle and at 25 °C.

Determination of Zeta Potential: The Zeta potential of the nanosuspensions was measured by

using an additional electrode in the same instrument used for particle size analysis (Malvern Zetasizer). Samples of formulations were diluted with water and placed in the electrophoretic cell. Each sample was measured three times at 25° C and the average values were calculated.

Drug Content: The prepared formulations were assayed individually for the content of the drug as follows; 10 mg of each sample was dissolved in 10 ml methanol. The drug content of each sample was assayed spectrophotometrically at λ_{max} 267 nm after proper dilution using methanol as a blank.

Lyophilization of the Selected ITZ Nanosuspensions:

The selected formulation (F3) was converted into powder by lyophilisation of nanosuspensions, first the nanosuspension was poured into ampoules and prefrozen in deep freezer at - 40°C for 24 hours (model FDU-7003, Korea), then the ampoules were transferred to glass flasks and the flask was attached to the vacuum adapter of lyophilizer.

Optimization of ITZ lyophilized nanoparticles

Morphology evaluation: The high resolution transmission electron microscope (TEM, JEM-1400, and Japan) was used to evaluate the morphology of the optimized formulation (F3) nanoparticles and compared with the morphology of the pure drug. A drop from each suspension was loaded onto a carbon coated copper grid. The excess suspension was removed immediately using filter paper. Negative staining using (2% w/v) aqueous solution of phosphotungstic acid was directly added to the grid and left for 45 seconds. Then, samples were dried for 1 hour. After drying, the grid was directly investigated and photographed using (TEM, JEM-1400, Japan)

Fourier transform infrared spectroscopy (FTIR):

The Fourier transform infrared (FTIR) spectra of pure ITZ, physical mixture and ITZ nanoparticles were recorded using FTIR spectrophotometer. Powders were mixed with potassium bromide (spectroscopic grade) and compressed into disks using hydraulic press before scanning from 4000 to 400 cm⁻¹.

Differential scanning calorimetry (DSC):

DSC was used to assess the crystallinity properties of the different materials and formulations. ITZ powder and ITZ nanoparticles (4-6mg) were sealed in the flat-bottomed aluminum pan of the differential scanning calorimeter (Shimadzu DSC-50, Japan). Data collection was carried out at a temperature range of 0–300°C, and the heating rate was 5°C/min under nitrogen gas at a flow rate of 25 ml/min. The transition and melting point

measurements were assessed using the device software.

X-ray powder diffractometry studies: The X-ray diffraction (XRD) patterns for pure ITZ and ITZ-nanoparticles were analyzed using an X-ray diffractometer (PANalytical). The freeze dried sample scanning was conducted over 2θ , ranging from 5° to 40° , at a voltage of 45 kV and a current of 40 mA.

In-Vitro dissolution Studies: The dissolution profiles of plain drug, selected nanosuspension formulation (F3) and the lyophilized nanoparticles were determined under sink conditions using USPXXX dissolution tester, apparatus (II). Briefly, an amount of samples equivalent to 50 mg of ITZ was placed into 900 ml 0.1NHCL containing 0.5% sodium lauryl sulfate (SLS) as dissolution medium maintained at $37 \pm 0.5^\circ\text{C}$. The paddle was made to rotate at 100 rpm [16]. At the predetermined time intervals an aliquots equivalent to 5 ml of the dissolution medium was withdrawn, filtered, and analyzed for ITZ content after proper dilution using spectrophotometrically at 267 (Jasco V530, Japan).

In-vivo drug absorption study

Selection of animals: Twelve albino male rabbits, weighing between 2.5 and 3 kg, were used in the in vivo study. Based on the results of the in-vitro studies, F3 was selected for the in vivo study. The rabbits were fasted overnight (>12 h) then divided into two groups, each containing 6 rabbits. Group I was administered the Sporanox[®] capsules 100 mg (reference) at a dose of 5 mg/kg. Group II was administered the nanoparticles (F3) at a dose of 5 mg/kg, each preparation was dispersed into 1ml of distilled water vortexing for 20 seconds immediately prior to dosing. Blood samples (0.5 ml) were collected before the administration of the drug and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10,12,16,24 and 36 h after the administration of the drug. Samples were stored at -20°C until analysis.

Extraction procedure: 0.5 ml from plasma was added to 50 μl (100 $\mu\text{g}/\text{ml}$) of internal standard (Ketoconazole), the sample is subsequently alkalinized with 1ml of 0.05M borate buffer (pH 10) and extracted twice with 5ml diethyl ether followed by centrifugation at 2000 rpm for 10 min. the upper organic layer was transferred into a fresh tube and evaporated under a gentle stream of air and the extraction residue was reconstituted with 500 μl of the mobile phase, vortex then filtered through filter disk and analyzed by HPLC [17].

Chromatographic conditions: The mobile phase consisted of acetonitrile: 0.05% diethylamine in

deionized water (6: 4, v/v). The pH of the mobile phase was adjusted to 6 with 30% acetic acid. After filtration through membrane filter, the mobile phase was degassed and pumped at flow rate 1 ml/min. The UV detector was adjusted at 263 nm and the element peaks were investigated using peak height ratio, all assays were performed at ambient condition.

Pharmacokinetic data analysis

The C_{max} and T_{max} were calculated using the plasma concentration-time curve in the WinNonlinTM Nonlinear Estimation Program. One way analysis of variance was employed to assess the significance of the difference between the T_{max} , C_{max} , $AUC_{(0-36)}$, $t_{1/2}$ and MRT data of ITZ from the tested nanoparticles formulation (F3) and the reference at a level ($p < 0.05$) using the SPSS program [18]. The level of absorption from the nanoparticles formulation (F3) relative to the reference was calculated as the relative bioavailability by using the formula given below:

Relative bioavailability (%) = $(AUC_{(0-36)} \text{ test} / AUC_{(0-36)} \text{ reference}) \times 100$.

RESULTS AND DISCUSSION

Particle size: The effects of type and amount of stabilizers on the particle size of nanosuspension were investigated to choose the optimum stabilizers for ITZ nanosuspensions. The results showed that the particle size reduced with the increasing of pluronic F68 concentration as the particle size of formula F3 was $(213.569 \pm 19.58 \text{ nm})$ compared with F9 that had particles size $(415.49 \pm 11.23 \text{ nm})$. The particle size increased with increasing the concentration HPMC of stabilizer where the particle size was $752.37 \pm 24.52 \text{ nm}$ at the 1:1 ratio (drug: polymer) compared to $424 \pm 18.59 \text{ nm}$ at the 4:1(drug: polymer). It should be mentioned that too little concentration of stabilizer results in high particle size values due to insufficient polymer to protect the nanoparticle surface leading to aggregation and/or agglomeration, while too much concentration of stabilizer results also in high particle size values due to Ostwald ripening [19]. The increase in particle size observed with the higher stabilizer concentrations for both the semi-synthetic polysaccharide based stabilizers (HPMC) and the synthetic linear polymers (PVP K17) might be due to Ostwald ripening resulting in agglomeration and consequently, higher particle size values. Moreover, both stabilizers yielding solutions with relatively high viscosity that could hinder particle attrition at the same milling energy [20].

The polydispersity index (PI): The polydispersity index is the measure of size distribution of the

nanoparticles. A low PI value indicates a narrow size distribution while a high value of PI indicates a broad distribution [21]. PI values were ranged from 0.230 -0.545 depending on formulation variables (Table 2) which indicates good uniformity in particle size distribution.

Zeta potential analysis: Zeta potential analysis was performed to investigate the surface properties and hence the stability of nanosuspension. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. Zeta potential is an important parameter for prediction of stability of nanosuspension. Zeta potential of formulation of F-1 to F-9 was observed between -8.73 to -18.35 mV, negative zeta potential is attributed to drug nanocrystals. Zeta potential of ITZ nanosuspension (F-3) was found to be -18.35 ± 2.55 mV (Table 2). Thus, it was concluded that the system had sufficient stability and selected for further studies. The value of zeta potential about ± 20 mV is sufficient to fully stabilize the nanosuspensions system [22].

Total drug content: Table 2, shows the drug content for the prepared formulations. The drug content for all formulations was satisfactory and was more than 85%, which indicates that loss of drug was lower during preparation process.

Morphology evaluation: The morphological characteristics were investigated using transmission electron micrographs (TEM). The results show large ITZ crystals within a micro range approaching 15 μ m in size (figure, 1a) and the nanoprecipitation method transformed these large crystals into small cubic nanoparticles (figure 1b).

Fourier transform infrared spectroscopy (FTIR): The IR spectra of raw ITZ, physical mixture and ITZ nanoparticles, are shown in figure 2. The main peaks of pure ITZ were observed at wave numbers 3126, 2969, 3069, 1698, 2822, 1511 and 1452 cm^{-1} . The absorption bands between 2800 cm^{-1} and 3200 cm^{-1} was attributed to the alkane, aromatic CH and amine groups and the sharp peak happened at 1699 cm^{-1} is due to C = O of the ITZ [23]. The IR spectra of the physical mixture and nanoparticles showed the characteristic peaks of ITZ and this suggested that there were no change in the ITZ chemical structure during processing.

Differential Scanning Calorimetry (DSC): The DSC thermogram of pure ITZ (figure 3) shows a characteristic sharp endothermic melting peak at about 167.12 $^{\circ}$ C with peak onset at 162.91 $^{\circ}$ C and

peak end at 167.17 $^{\circ}$ C and the heat of transition was (-5.06 J/g). The thermogram of ITZ nanoparticles (F3) shows endothermic transition at 155.74 $^{\circ}$ C which is close to the expected value for the drug. The presence of the small endothermic peak of ITZ nanoparticles and the shift in the ITZ peak to a lower temperature in the nanoparticles compared to pure ITZ might be due to smaller ITZ crystals [24].

X-ray diffraction: Powder X-ray diffraction was performed to analyze the nanoparticles. The X-ray diffraction pattern of ITZ showed sharp diffraction peaks suggesting that both ITZ as received and nanoparticles were crystalline (figure 4). The sharp peaks for raw ITZ indicate the crystalline state of raw ITZ. XRD results showed that the sonication and freeze drying process did not affect the ITZ crystalline state. X-ray diffractometer confirmed the crystalline nature of ITZ nanoparticles [25, 26].

In-vitro dissolution: The most important feature of nanoparticles is the increase in the dissolution rate not only because of increase in surface area but also because the use of hydrophilic surfactant. The in-vitro dissolution of ITZ was carried out for both of the selected nanosuspension (F3) and the lyophilized nanoparticles, and was compared to that of the pure drug powder (figure 5). The cumulative percentage of the drug dissolved was 85.64% and 93.81% at 60 min for selected nanosuspension and the lyophilized nanoparticles respectively, while the cumulative percentage of the pure drug was 35.52 at 60 min %.

In-vivo pharmacokinetics study: F3 was selected for the pharmacokinetic study according to the physicochemical characterization. The mean plasma concentration–time data of ITZ following the administration of the marketed Sporanox[®] (100mg capsules) and formula (F3) is shown in figure 6. There was a statistically significant difference in the T_{max} , C_{max} , $AUC_{(0-36)}$ and MRT data between the market formulation and the nanoparticles (F3). The C_{max} value of ITZ nanoparticles was significant ($p < 0.05$) higher than market formulation (Table 3), T_{max} significant decreased ($p < 0.05$) for ITZ nanosuspension (2.167 ± 0.389 hour) compared to Sporanox[®] capsules (4.667 ± 0.984 hour). The $AUC_{(0-36)}$ value of ITZ nanoparticles after oral administration was almost 2 folds higher than those obtained of the marked formulation. Possible explanation for significantly higher $AUC_{(0-36)}$ ($p < 0.05$) is due to the great increase in dissolution which is attributed by the presence of surfactant and smaller particle size, and thus increased the relative bioavailability to 180.6% compared to the market formulation.

CONCLUSION

Nanoprecipitation method was successfully employed to produce stable ITZ nanosuspension by using the proper stabilizer (pluronic F 68) and surfactant (tween 80). ITZ nanoparticles was easy prepared by lyophilization technique and the

dissolution study shows that the nanosuspension formulation and the lyophilized nanoparticles give higher drug release compared to the pure drug. Nanoparticles consequently represent a promising new drug formulation for oral drug delivery of the drug and enhancement the oral absorption of ITZ.

Table 1. Nanosuspension formulations for optimization of stabilizing agent and its proper ratio

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
ITZ	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg
HPMC	10 mg	-----	-----	5 mg	-----	-----	2.5 mg	-----	-----
PVP K 17	-----	10 mg	-----	-----	5 mg	-----	-----	2.5 mg	-----
Pluronic 68	-----	-----	10 mg	-----	-----	5 mg	-----	-----	2.5 mg
Tween 80	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml
Ethanol	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml
Water	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml

Table 2. Physicochemical characterization of Itraconazole nanosuspensions

Formulation	Particle size (nm) ± s.d	Polydispersity index ± s.d	Zeta potential (mV) ± s.d	Total Drug Content (%)
F1	752.37±41.25	0.412±0.20	-10.36± 2.96	86.43
F2	623.86±22.47	0.289±0.17	-11.38± 3.81	92.58
F3	213.569±19.58	0.219±0.19	-18.35± 2.55	94.67
F4	623.6±38.11	0.461±0.20	-10.5 ± 1.94	88.38
F5	538.23±25.64	0.287±0.16	-13.38± 2.87	90.89
F6	341.43±29.26	0.319±0.11	-16.64± 4.11	92.95
F7	471.64±33.45	0.398± 0.20	-8.42± 3.35	87.84
F8	401.45±28.08	0.351±0.14	-13.22± 2.11	91.638
F9	415.49±11.23	0.257 ± 0.09	-15.92± 4.21	89.25

Table 3. Pharmacokinetic parameters after oral administration of Itraconazole formulations to rabbits (n =6).

Pharmacokinetic parameters	Formulations	
	Marketed formulation (Sporanox® capsules)	Nanoparticles (F3)
C _{max} (ng/ml)	95.25±18.972	220.605±31.177
T _{max} (hr)	4.66±0.984	2.166±0.389
AUC ₍₀₋₃₆₎ (ng.hr/ml)	1197.5675±131.42	2162.752±176.98
% Relative Bioavailability	-	180.61 %
*t _{1/2} (hr)	18.78±2.95	20.62±2.48
MRT (hr)	19.8798±1.63	24.5.32±2.88

*Data expressed as mean ± standard deviation.

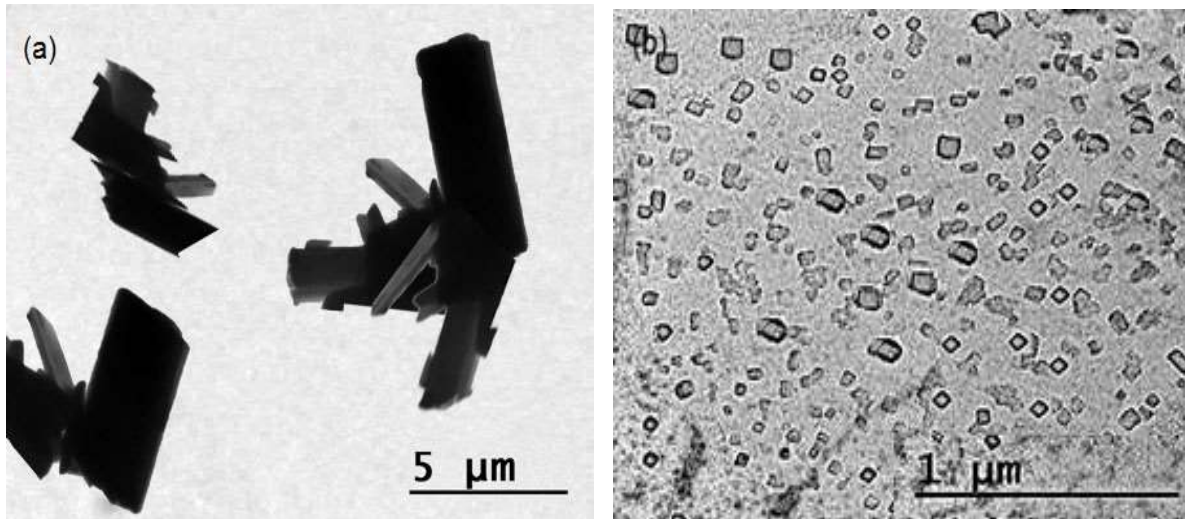


Figure 1: Transmission Electron Micrographs (a) pure Itraconazole and (b) Lyophilized nanoparticles (F3)

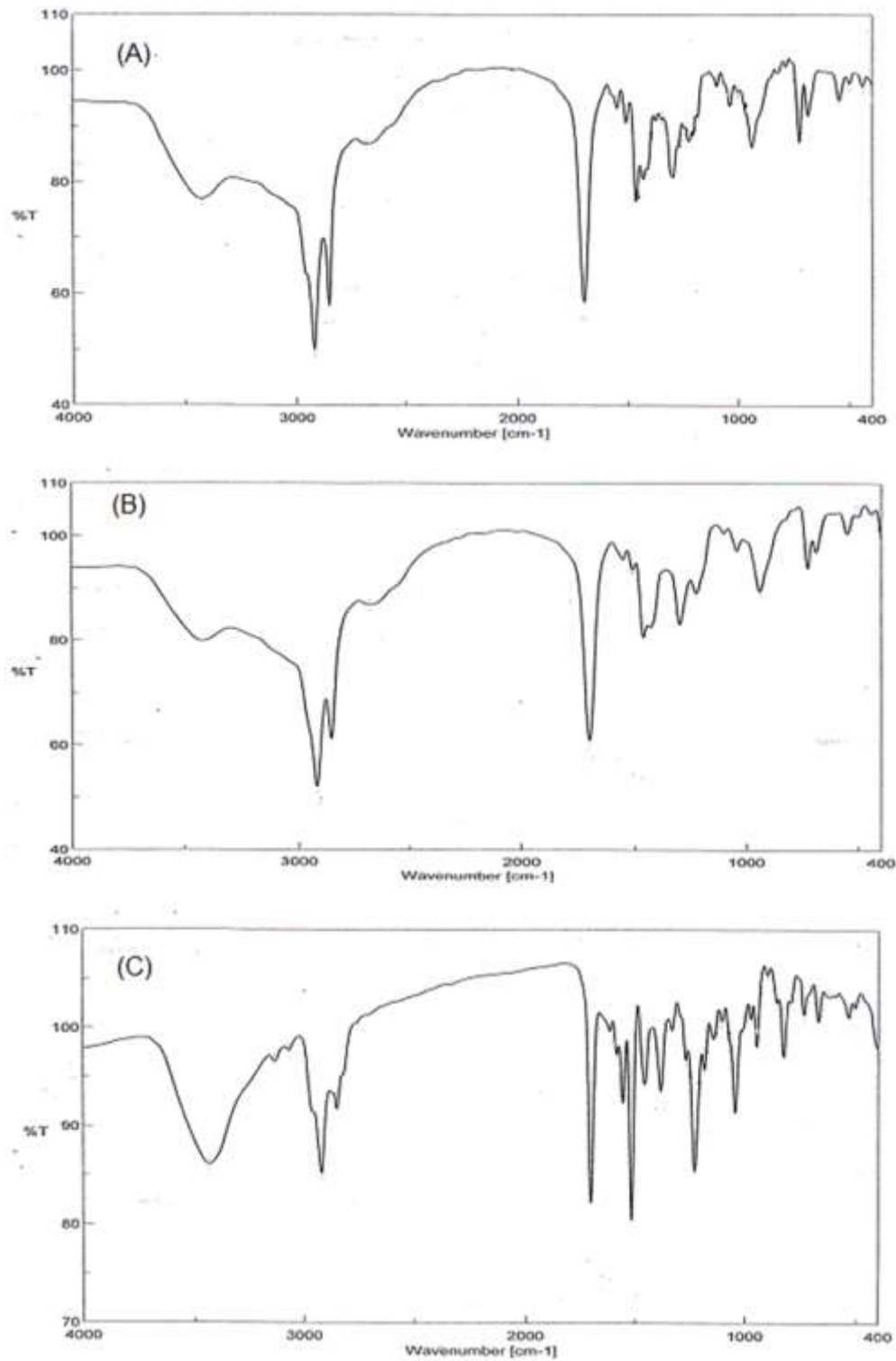


Figure 2: FTIR spectra (A) pure Itraconazole ,(B) Physical mixture and (C) Lyophilized nanoparticles (F3)

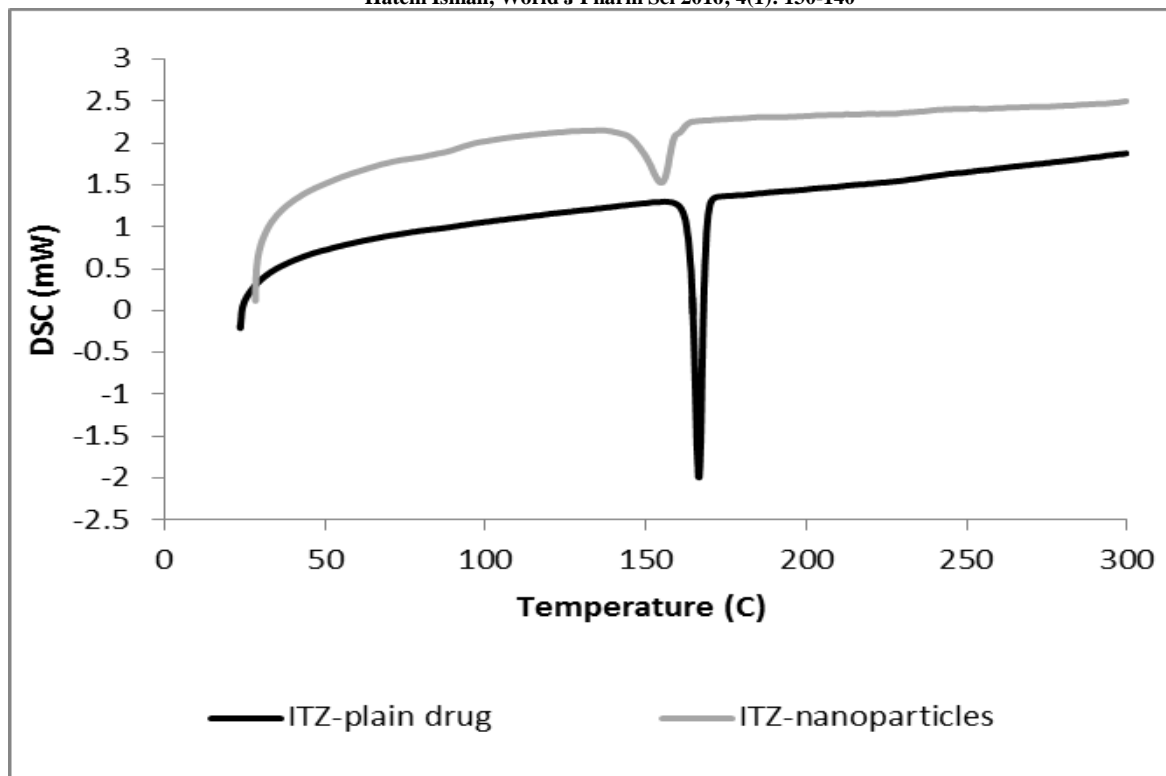


Figure 3: DSC thermogram of pure Itraconazole and lyophilized nanoparticles (F3)

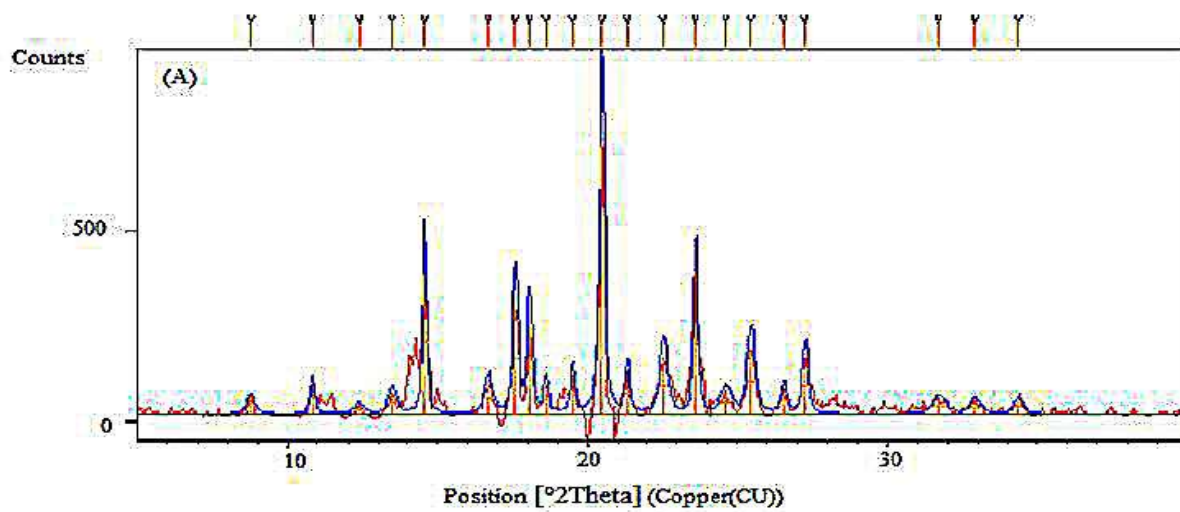


Figure 4 (A): X-ray diffraction of pure Itraconazole

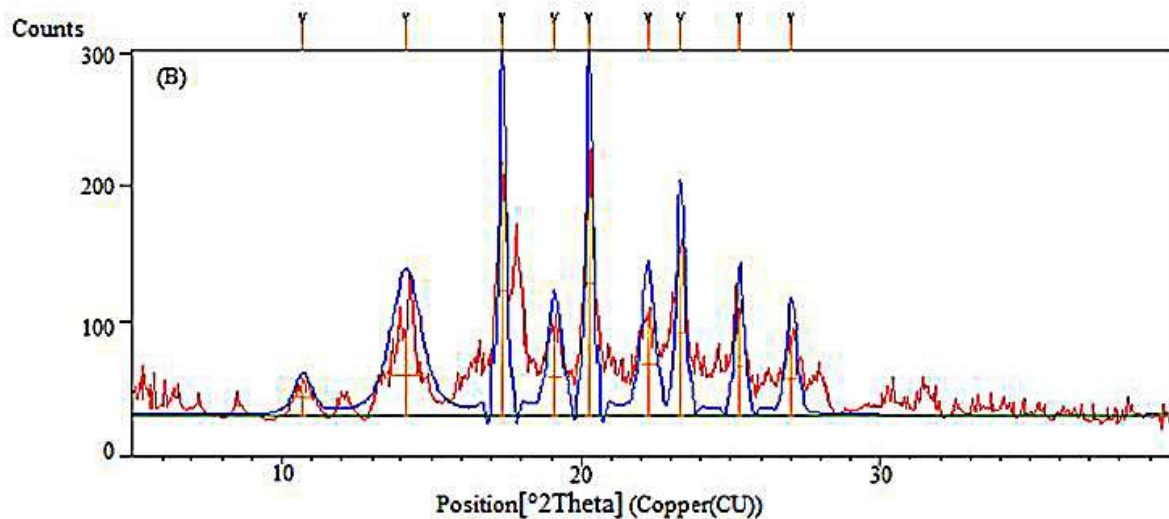


Figure 4 (B): X-ray diffraction of f lyophilized nanoparticles (F3)

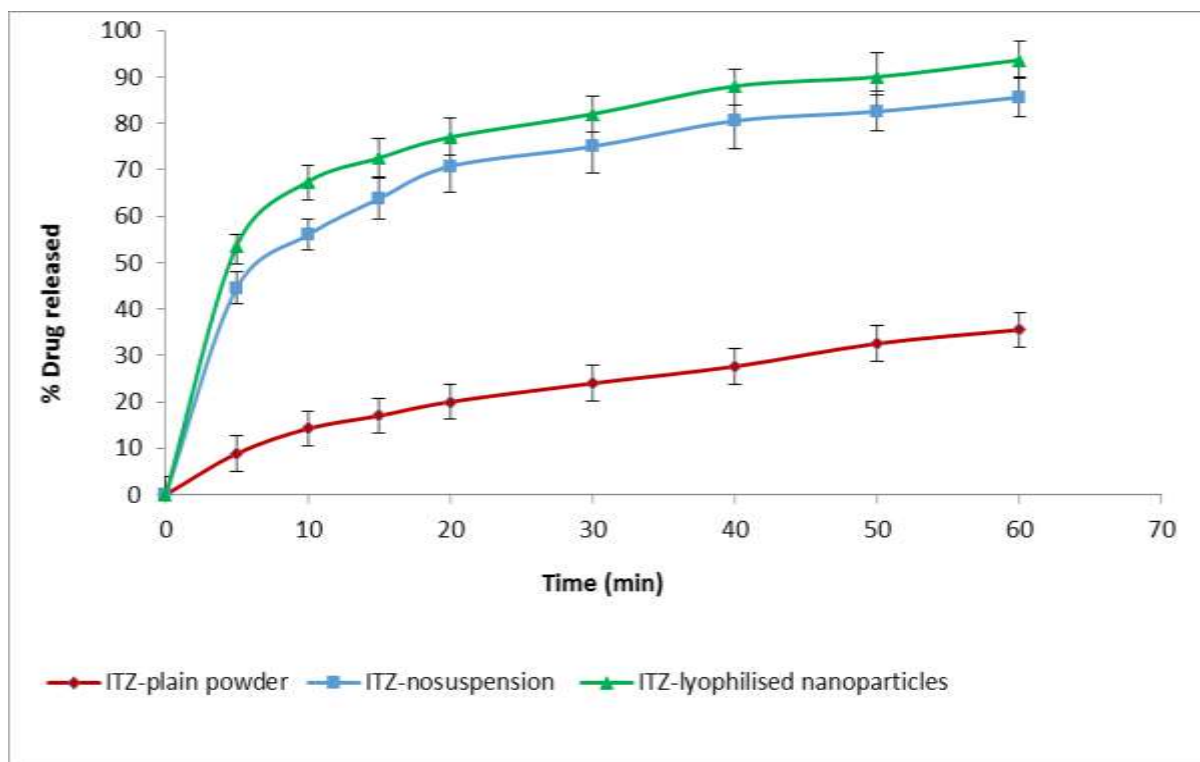


Figure 5: Dissolution profile for pure drug, nanosuspension formulation (F3) and ITZ-lyophilized nanoparticles (F3) [mean ± SD (n=3)]

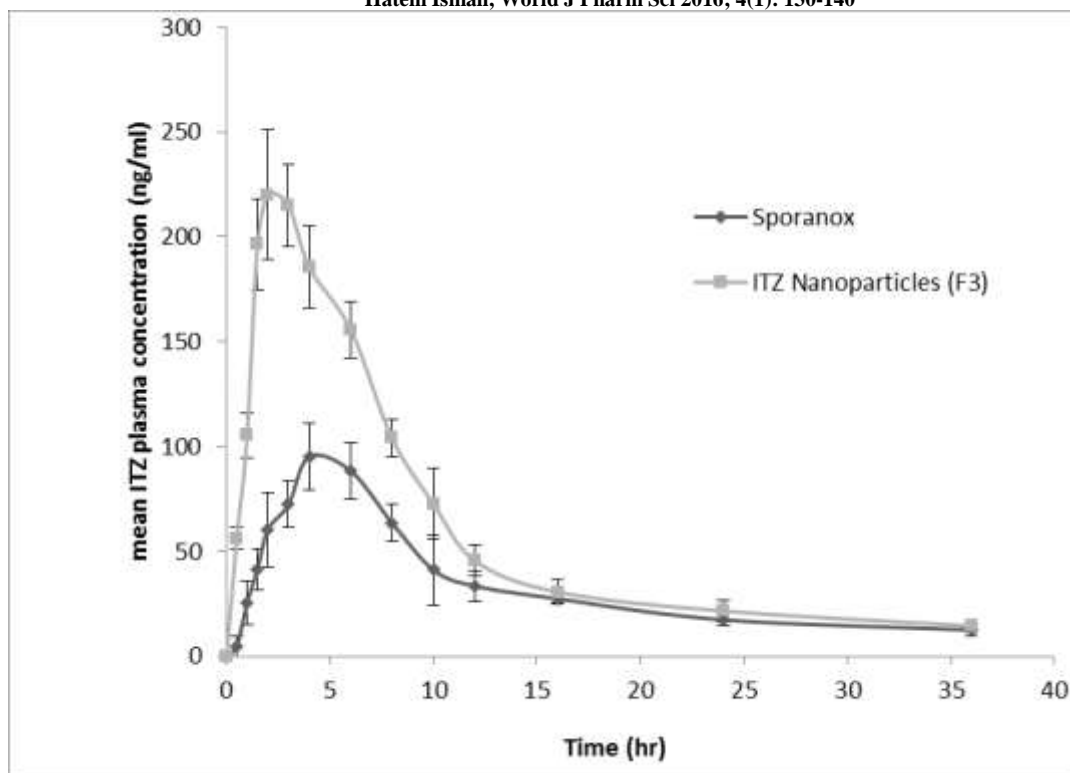


Figure 6: Plasma concentration of Itraconazole following the administration of Sporanox® capsules and ITZ nanoparticles (F3), data represent the mean values of (n=6 ± S.D).

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