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Preparation and characterization of Itraconazole solid dispersions for improved oral bioavailability

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Abstract: The main objective of the present study is to improve the dissolution and in vivo bioavailability of Itraconazole by preparing the solid dispersion using lipid materials as inert excipients. The drug encapsulated dispersions are spray dried and are duly characterized for drug content, particle size distribution, drug morphological conversion, drug compatibility with the excipients, *in vitro* dissolution and *in vivo* bioavailability. The drug content in the prepared dispersions is between 80.0% and 95.0% (w/w) of the theoretical values and the mean volume diameter (VMD) of the particles collected from drying chamber and cyclone are found to be 21.65 & 33.66µm, respectively. The DSC thermo grams have indicated the morphological conversion of itraconazole to amorphous form. The saturation solubility for itraconazole in the spray dried formulation is 5.2 and 2.6 times higher to the plain drug and spray dried drug, respectively. The dissolution of itraconazole in acetate buffer pH 1.2 is 69% in the solid dispersion formulation where as it is only 17% in the plain drug in 60 mins. And the enhancement of dissolution is 4.07 times higher to plain drug after 60mins. The formulations have demonstrated the significant improvement of bioavailability (AUC=14384ng/h/ml) compared to plain drug suspension (AUC=4384ng/h/ml). These results demonstrated the efficacy of solid lipid dispersions for the enhancement of oral bioavailability of itraconazole by increasing its aqueous solubility
Key words: Itraconazole, Dispersion, Bioavailability, Spray drying, Dissolution, Oral.

1.0 Introduction

Itraconazole (ICZ) is a broad spectrum antifungal agent and belongs to trizole group indicated in the treatment of local and systemic fungal infections[1]. Itraconazole is weakly basic (pK_a=3.7) and highly hydrophobic. Classified as Biopharmaceutical Classification System II drug, ICZ has poor aqueous solubility and poor dissolution in gastrointestinal tract. Because of poor aqueous solubility, ICZ on oral administration results in poor bioavailability and inter individual variations in the plasma drug concentrations. And ICZ has the characteristic of pH dependent solubility having highest solubility at acidic side (4µg/ml) compared to basic pH (1ng/ml). However, because of highly liphophilic nature (log P= 6.2) ICZ can easily penetrate into intestinal membrane[2]. This indicates the poor aqueous solubility is the main reason for lower plasma concentrations. In the recent times numerous articles have been published explaining the strategies such as preparation of amorphous ICZ[3], cyclodextrin complexations[4], solid dispersions with eudragits[5], particle engineered compositions[6] to improve the aqueous solubility of ICZ and to enhance the oral bioavailability. Solid dispersions proven to be efficient strategy in enhancing the oral bioavailability of liphophilic drugs. Solid dispersions have been defined as "the dispersion of one or more active ingredients in an inert excipient or matrix" where the active ingredients could exist in finely crystalline, solubilized or amorphous states. Different polymers have been utilized in the preparation of solid dispersions as inert matrices, however met with some challenges such as stability, process etc. These challenges created the way to prepare the solid dispersions with alternative materials such as lipids. These lipid formulations range from

simple solutions of drugs in dietary triglycerides to the use of complex mixtures of triglycerides, partial glycerides, surfactants, co surfactants and co solvents to solubilize the drugs[7-8]. However the work that was carried out in lipid mediated solid dispersions involves the dispersion of drug in the molten lipid and filled into hard gelatin or soft gelatin capsule. To make a solid oral dosage form, the prepared solid dispersions shall have suitable flow property. Spray drying is widely used technique in developing free flowing powders from aqueous or non aqueous suspensions and solutions[9].

In the present paper we discuss the preparation of ICZ loaded solid dispersions, characterization and in vivo evaluation of the developed formulation. The lipid excipients, Polyglycolized glycerides (Gelucire 50/13) and glyceryol dibehenate (Compritol), have been utilized for the preparation of dispersions. Gelucire and compritol are dissolved in dichloromethane (DCM) and spray dried to get fully dried particles. The prepared dispersions are duly characterized by DSC for morphological conversion and the in vitro release study was conducted. Finally the formulations are evaluated for their in vivo absorption by orally administering the formulations to the rats.

2.0 Materials and Methods

2.1 Materials: Gelucire 50/13 (Stearoyl macrogoglycerides) and Glyceryl dibehenate (Compritol 888 ATO) were generous gifts from Gateefosse, india. All HPLC and analytical grade chemicals were purchased from Merck, India.

2.2 Preparation of Itraconazole loaded solid dispersions

The lipid matrix consisting of gelucire and compritol at a weight ratio of 1:3 was dissolved in DCM and ICZ is dissolved in this solution at a final concentration of 1:3, drug to lipid ratio. The lipid and drug dispersed solution was fed into a spray drier (Labultima, Mumbai) with a co-axial nozzle with co current flow. The total concentration of the solution was 5 w/v%. The conditions, that are maintained during spray drying are as follows: Inlet temperature 50^{o}c, Outlet temperature 40^{o}c, feed rate 3ml/min, atomization pressure 2.5kg/cm^2 and aspiration of 25m^3/h. The dried drug loaded dispersions (F1) are collected from drying chamber and cyclones and stored in desiccated environment until further study. Plain ICZ was dispersed in DCM and spary dried using above mentioned conditions (F2).

2.3 HPLC method development for Itraconazole estimation

ICZ is estimated using a validated RP-HPLC method by Agilent make model 1200 series. The mobile phase is a filtered and degassed mixture of 0.1m sodium dihydrogen phosphate solution and Acetonitrile in the ratio 38:62(v/v) and the separations were performed with L1-Packing (Zorbax XDB, C18-150 x 4.6mm, 5µm) column. The chromatographic conditions were as follows: The nanometers is set to 263nm, flow rate is 1.0 ml/min, injection volume 100µL with a run time of 10 minutes. System is found to be suitable after injecting blank followed by standard (six times) and evaluating the %RSD (<2.0%) and USP tailing factor (<2.0).

2.4 Drug content estimation

The content of ICZ in the solid dispersion (F1) sample was determined using HPLC. A 10 mg sample was dissolved in 10 mL of acetonitrile and vortexed well. The solutions were filtered through a membrane filter (0.45 mm) and suitably diluted with mobile phase before injecting to the HPLC.

2.5 Saturation Solubility

To evaluate the increase in solubility of ICZ after the preparation of solid dispersion, saturation solubility measurements were conducted for formulations F1 & F2. The impact of spray drying on the solubility enhancement was studied by taking plain ICZ as a control. The known excess amount of ICZ was added to 10 mL of pH 1.2 acetate buffer. Samples were rotated at 20 rpm in a water bath (37± 0.5^{o}C) for 48 hours. The samples were then filtered, suitably diluted, and analyzed by HPLC.

2.6 Laser Diffraction Particle Size Analysis

Particle size distribution was measured using a laser diffraction size analyzer (HELOS/BF-R5, Sympatech, Germany). Samples were suspended in water and two to three drops of isopropyl alcohol added to the dispersion and treated by ultra sonication at 50% amplitude. The particle size and distribution was measured at a measurement range

2.7 Differential Scanning Calorimetry (DSC):

DSC studies were conducted using a TA instrument, Model Q200 equipped with a with RCS-90(-90°C to 450°C) cooling unit. DSC was performed with 2mg sample in Tzero pan-Aluminium, encapsulated with Tzero lid-Aluminium by T zero press. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 mL/min. Samples are heated at a temperature range of 0 to 300°C with ramping at 10°C.

2.8 Infrared Spectroscopy (IR):

Infrared spectra's were obtained for plain itraconazole, gelucire, compritol and spray dried formulation (F1) for evaluating the chemical compatibility of ICZ with the excipients used in the formulation development. Spectra's were taken after

preparing the pellet with 2-3 mg of sample with potassium bromide using FTIR spectrometer (Jasco) and the sample was scanned from 4000-400cm^{-1}.

2.9 In vitro dissolution:

The dissolution rate of ICZ from the prepared dispersion (F1) was measured in a Disso-2000 model dissolution test system (Labindia, India) using simulated gastric fluid (SGF) without pepsin at pH 1.2 and USP apparatus II (paddle) method. The drug dispersed dispersions are filled into hard gelatin capsule equivalent to 30mg of ICZ. The equivalent plain ICZ was filled into capsules along with mannitol as an inactive excipient (F3). In each dissolution vessel, drug filled capsules were added to 900 mL dissolution medium. Bath temperature and paddle rotation speed were maintained at 37°C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After collection of 90min sample, recovery study is conducted by stirring the paddle at 200rpm for 5min and sample is collected. Samples are filtered through filters (10µm) and analyzed using HPLC.

2.10 In Vivo bioavailability study

Male wistar rats (Suven life sciences, Hyderabad, India) weighing 220-250gm are used for the study. The rats were housed in stainless steel cages and kept on a 12 hr light/dark cycle. On the day of experiment, animals were randomized and divided into groups of four and kept for fasting for 12hrs. All animal studies conducted are approved by local animal ethical committee.

The formulations are prepared for administration by dispersing the ICZ solid dispersion (F1) in potassium in phosphate buffer solution (pH 7.4; 0.16M) and administered orally at a dose of 15mg/kg body weight. The plain ICZ is administered as a suspension (F4) in phosphate buffer solution (pH 7.4; 0.16M) and at a dose of 15mg/kg body weight. Blood samples were withdrawn from the animals after a period of 15, 30, 60, 90,120min post administration of the dose. The blood samples were collected into glass tubes containing disodium-EDTA and Plasma was separated by 1500g centrifugation at 4^{o}c. The plasma samples were transferred into plastic tubes and stored at -80^{o}c until further analysis was done. The drug levels in plasma are estimated by HPLC after extracting the drug from plasma. The drug extraction from plasma includes the addition of 300µl plasma into a 15 mL centrifuge tube. Then 50 µl of 0.3N Barium Hydroxide solution and 50 µl of 0.4N Zinc sulphate solution is added and vortexd for 90 sec. Further 1 mL of Acetonitrile is added and vortexd for 90 sec. The solution is centrifuged at 3000 RPM for about 10 min. and 1 mL of supernatant is removed, solvent is evaporated by nitrogen gas purging. The residue is reconstituted with 500µL of mobile phase and centrifuged at 3000 RPM about 10 min. And the supernatant is injected into HPLC.

3.0 Results and Discussion

The preparation of solid dispersion is a process in which the amorphous active moiety is molecularly dispersed in the inert lipid matrix. It is prepared by dissolving the lipids and active drug in common solvent and spray drying. The spray drying process conditions are chosen so that the solvent rapidly evaporates from the droplets to rapidly solidify the lipid and drug mixture, trapping the drug in amorphous form. In the present study, gelucire 50/13 and glyceryol dibehenate are taken as lipid matrix. The Gelucire 50/13 is a surface-active excipient that can solubilize poorly soluble drugs[10]. However spray drying of gelucire alone is problematic since it forms a sticky and tacky mass in the drying chamber because of its low melting point. Hence gelucire in combination with high melting lipids such as glyceryol dibehenate is designed. Upon spray drying solid powder with good flow property was obtained with a yield of 75%. Selection of the suitable solvent and spray drying conditions results in the formation of homogenous solid dispersion.

3.1 Solid state characterization

The soild state properties of prepared solid dispersions such as particle size, morphology of drug are very much critical in the successful development of solid dispersions for the enhancement of aqueous solubility. Hence the prepared spray dried solid dispersions are evaluated for particle size and distribution and drug morphology. The size of the particle significantly influences the dissolution. And it is generally regarded as lower the particle size the higher surface area and results in higher dissolution[11]. The particles are separated based on their size and collected from drying chamber and cyclone in spray dryer. The size of particle is evaluated by laser diffraction and was represented in table 2 and Fig.2. The mean volume diameter (VMD) of the particles collected from drying chamber and cyclone are found to be 33.66µm and 21.65µm, respectively. And the 90% of particles collected from cyclone and drying chamber are below 40.06 µm & 72.65µm, respectively. The size of the particle is precisely controlled by atomization pressure and feed rate during the spray drying process.

3.2 Differential Scanning Calorimetry (DSC)

DSC thermograms are obtained for ICZ, gelucire, compritol and for solid dispersion (F1) and are displayed in Fig.2. Pure ICZ has shown well defined endothermic peak at 168.11^{o}c corresponding to the melting point of crystalline drug. Likewise the lipid excipients have shown endothermic peaks at

43.42°c and 71.82°c for gelucire and compritol, respectively, representing the melting points. However in the thermogram of the spray dried solid dispersion, the endotherm peak of drug disappeared and instead new peak was observed at 158.3°c. However the endothermic peaks of gelucire and compritol remains same. The significant reduction in the melting point of the ICZ can be attributed to the morphological conversion of ICZ from crystalline to amorphous form.

3.3 Infrared Spectroscopy:

To evaluate the chemical compatibility between the Itraconazole and excipients, IR analysis was performed and represented in Fig.3. The IR spectra's of plain Itraconazole showed characteristic peaks at 400-1800cm^{-1}. They might have arisen from the stretching and vibrations of functional groups such as –C=C- of aromatic groups. A peak observed at 1600-1800 cm^{-1} can be attributed to –C=O stretching and vibration, whereas peaks for alkane and amine groups were noticed at 2800-3200 cm$^{-1.}$ Peaks of lipid carriers, gelucire & compritol, have shown significant broadening O-H stretching vibrations peaks at between 280—3200 cm^{-1} representing the characteristic peaks of lipids. The same peaks were seen in the spectra of formulation also. Also the major peaks observed for Itraconazole before and after the preparation of solid dispersion formulation at 400-1800cm^{-1} were almost superimposable. This suggested the absence of any significant interactions between Itraconazole and excipients used to make the solid dispersion formulation.

3.4 Drug content and Saturation solubility

HPLC analysis was used to estimate the drug content in the formulations. The obtained values for the formulation (F1) were between 80% and 95% (w/w) of the theoretical values.

The saturation solubility study is conducted for the plain drug, spray dried drug and spray dried formulation in phosphate buffer pH 7.4. After 48hrs of incubation the solubilized drug is evaluated by HPLC and values represented in the table 1.The saturation solubility for ICZ in the F1 is 144.5±10.5 µg/ml and is 5.2 and 2.6 times higher to the plain drug (27.65 µg/ml) and F2 (54.8 µg/ml) formulations, respectively. The higher solubility in the formulation is because of the morphological conversion of the drug and also attributed to the improvement of wetting of the drug, reduced particle size and localized solubilization by lipid carriers.

3.5 In vitro dissolution:

To asses the performance of developmental formulations prior to in vivo testing, in vitro dissolution testing has routinely conducted under sink conditions. The dissolution profile of the F1 and F3 was shown in the Fig.4. The release of ICZ from the F1 is steepest initial slope and the dissolution rate was higher compared to F3 in all time points. The enhancement of dissolution was approximately 3-4 times higher until 60 mins compared to F3. The percent drug release in F3 formulation in 60 mins is 51% whereas it is only 19% in the plain drug formulation. It is assumed that there are two mechanisms responsible for dissolution of ICZ. They are drug controlled and carrier controlled dissolution. Because the ICZ in solid dispersion is in amorphous form and hence the dissolution was more compared to plain drug. And by means of spray drying more precise particles can be prepared and the produced smaller particles enhance the dissolution by increased surface area. And the spray dried particles could improve the wettability of the drug and localized solubilization by the lipid materials in the diffusion layer more efficiently[12].

3.6 In Vivo bioavailability study

The efficacy of the F1 in the improvement of oral bioavailability of ICZ was evaluated after administering the dose to the rats. The plain drug suspension was prepared (F4) and administered to the rats for comparative evaluation. The tested formulations (F1 & F4) were dispersed in pH 7.4 phosphate buffer and administered orally. All the dosage forms were well tolerated and no obvious side effects were observed. After dosing, plasma samples were analyzed by HPLC for ICZ levels and drug plasma concentrations were as a function of time was shown in Fig.5. The plasma profiles were analyzed by non compartmental analysis for extravasucular administration to determine the appropriate pharmacokinetic parameters of administered formulations and represented in table 3. Statistically significant differences were observed for AUC (0-inf) values indicating that developed solid dispersion formulation has improved the oral bioavailability of ICZ and suggested that large concentrations of drug were available for absorption.

The F1 has shown increased C_{max} value compared to F4. The C_{max} values found to be 43.4ng/ml whereas the F4 has shown only 23.72ng/ml which is 1.58 times lesser to the formulation F1. And the AUC (0-inf) values are 3.2 times higher in formulation F1 compared to F4 (14384 vs 4384 ng/h/ml). However in the F1 administered formulations the Tmax found to be below 30min whereas it is below 15min in the case of F4. The increase in the C_{max} and AUC (0-inf) in the F1 compared to F4 can be mainly attributed to the enhancement of aqueous solubility and dissolution properties.

4.0 Conclusion

In the recent years the utilization of lipid excipients in the enhancement of oral bioavailability of poorly soluble drugs has become prominent. In the present work the solid dispersion with lipid excipient for an aqueous insoluble drug, itraconazole, is prepared by spray drying. The *in vitro* dissolution tests, *in vivo* bioavailability studies proved the efficacy of the formulation in the aqueous solubility and oral bioavailability enhancement compared to plain drug. The enhancement of aqueous solubility and oral bioavailability can be attributed to the factors such as reduced particle size, amorphous form of drug, increased solubility of drug by lipids and minimization of hepatic metabolism by lymphatic transport. Hence it is concluded that the oral bioavailability of poorly soluble drugs can be increased by preparation of solid dispersions by spray drying using lipid excipients.

Table 1: Saturation solubility study of Itraconazole formulations tested in pH 1.2 Acetate buffer at 37± 0.5^{o}C. (Mean ± SD)

Formulation	Saturation solubility (µg/ml)
Plain Itraconazole	27.65±1.3
F1	54.8±4.8
F2	144.5±10.5

Table 2: Particle size analysis data of the lipid dispersions (F1) collected from Cyclone and drying chamber after spray drying

Fraction	X10	X50	X90	VMD (µm)
Cyclone	6.97	16.83	40.06	21.65
Drying chamber	6.41	25.78	72.65	33.66

Table 3: Mean Pharmacokinetic parameters for Itraconazole formulations in plasma after oral administrations to the rats

Parameter	**F1**	**F4**
Cmax (ng/ml)	43.4	27.32
Tmax (min)	<30	<15
AUC (0-t) (ng.hr/ml	8702.3	3301.
AUC (0-inf) (ng.hr/ml	14384	4384

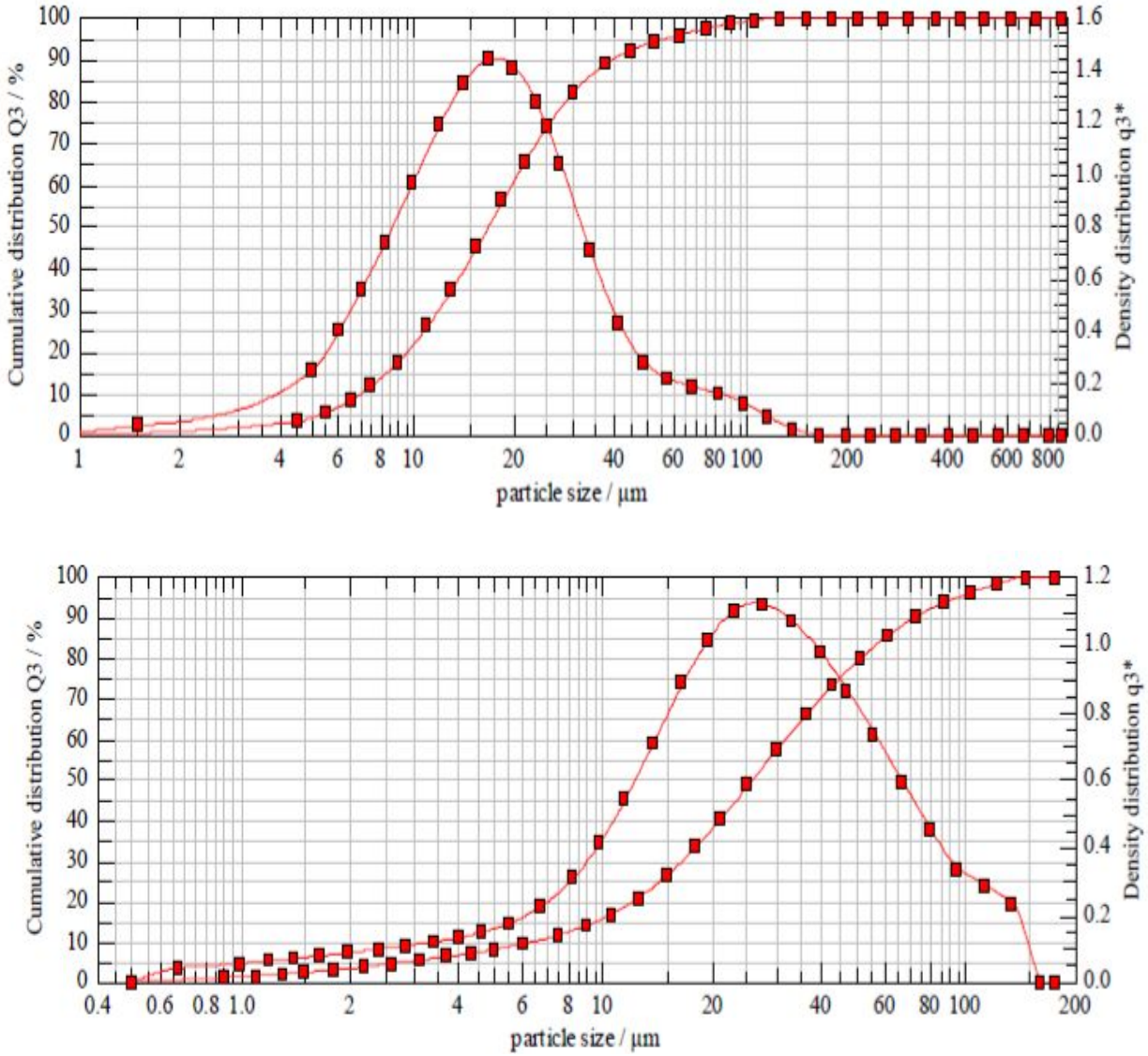
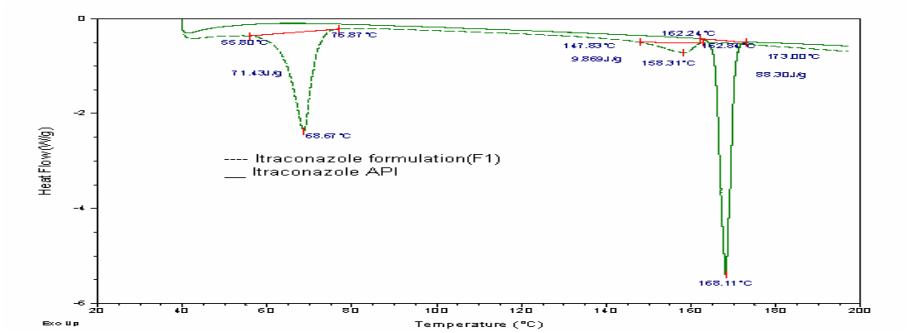
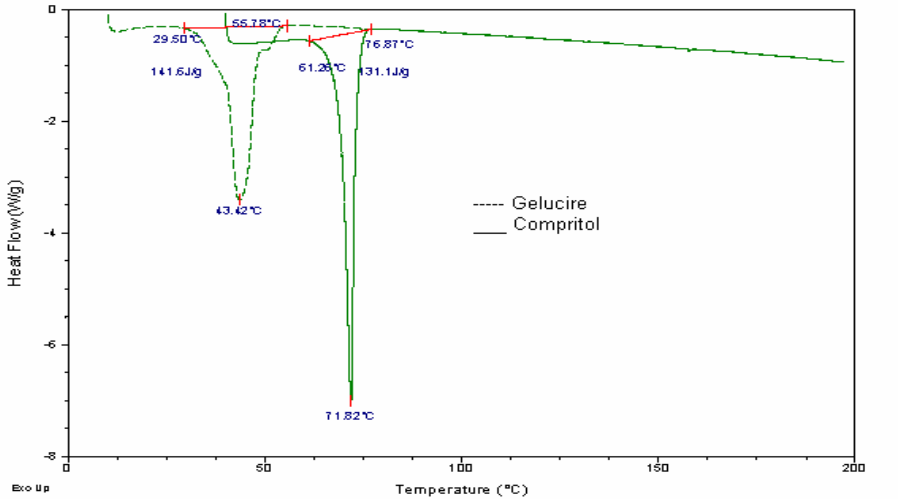


Fig 1: Particle size distribution of lipid solid dispersion (F1) prepared by spray dried process. A) Particles collected from cyclone B) Particles collected from drying chamber



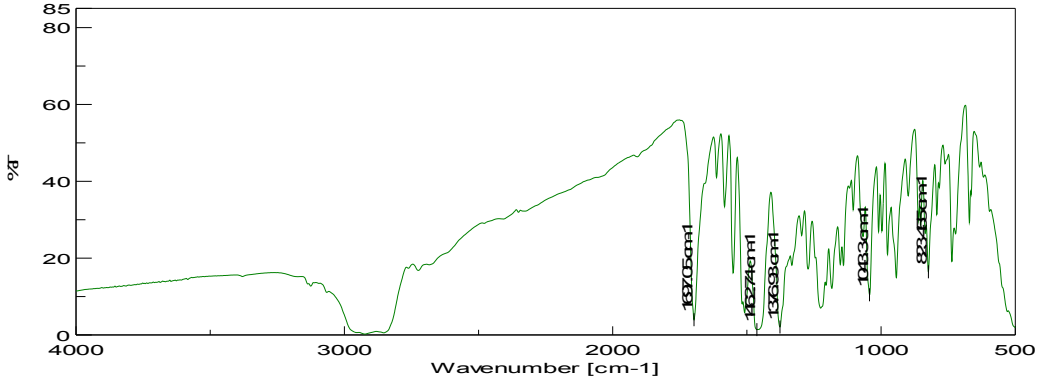
(A)



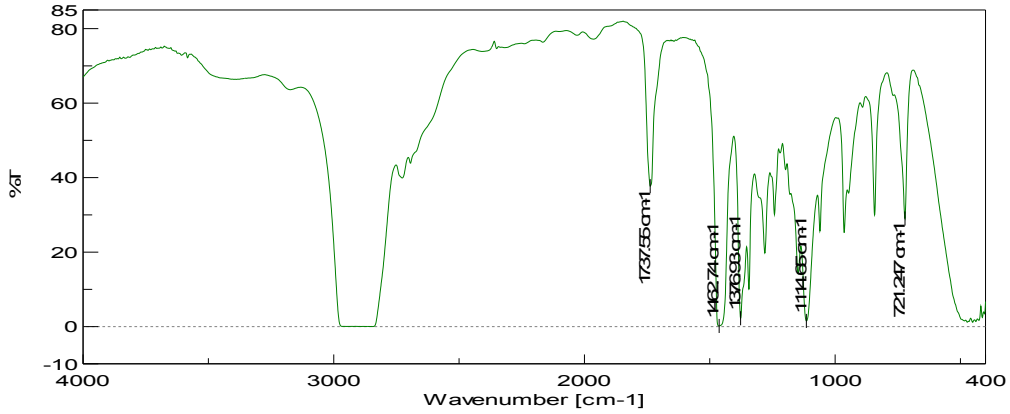
(B)

Fig.2: Differential Scanning Calorimetry (DSC) thermograms of Itraconazole, gelucire, compritol and Itraconazole loaded solid dispersion. A)Thermgrams of Itraconazole API & F1 B)Thermograms of Gelucire & Compritol

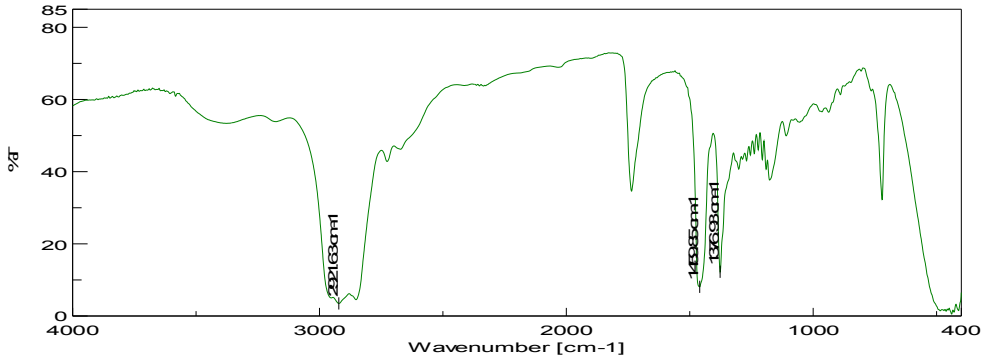
A) Itraconazole



B) Gelucire



C) Compritol



D) Formulation F1

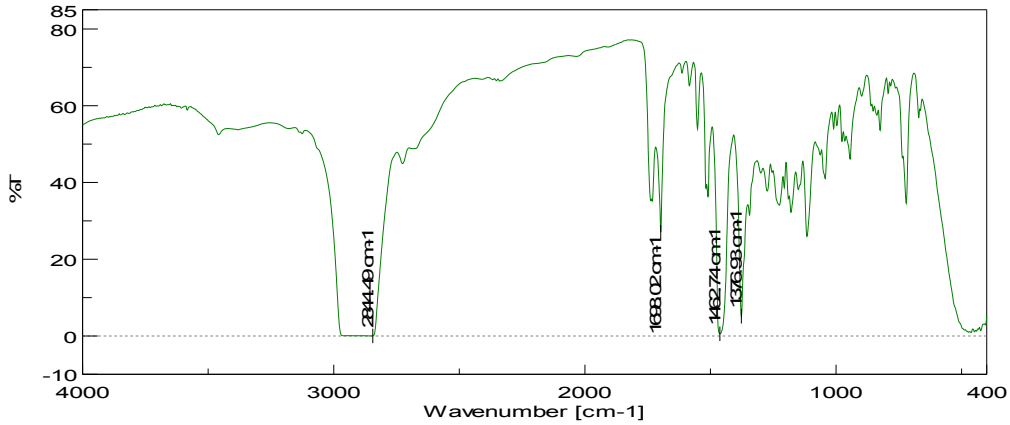


Fig.3: Infrared spectra's of Itraconazole (A), gelucire (B), compritol (C) and formulation-F1(D)

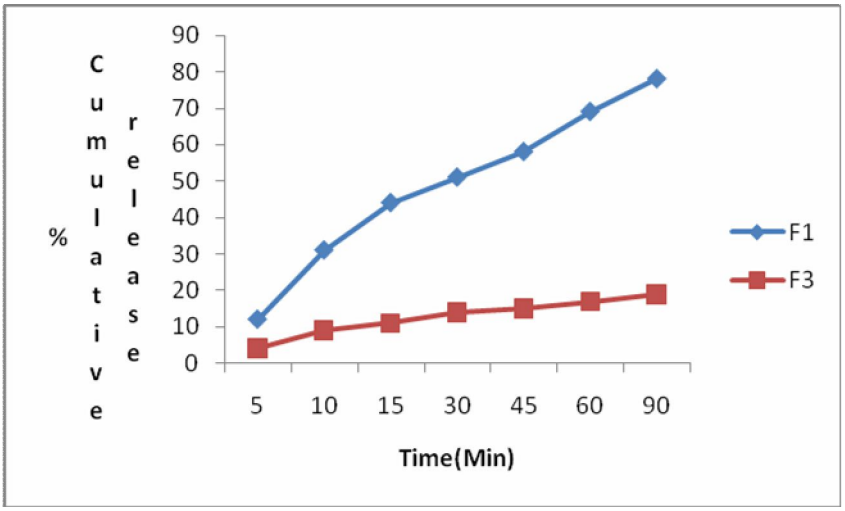


Fig.4: Dissolution profile of spray dried formulation (F1) and plain Itraconazole (F3) carried in acetate buffer pH 1.2.

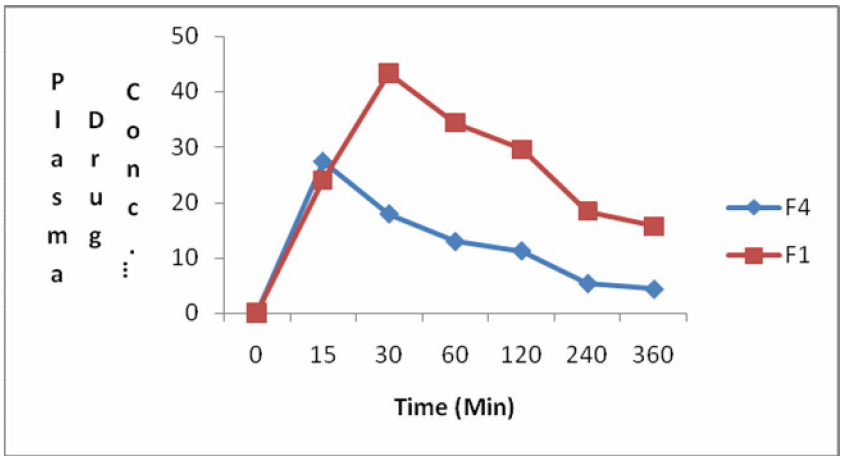


Fig. 5: Itraconazole plasma concentrations in rats after oral administration of formulations (F1) and plain drug suspension (F4).

5.0 Acknowledgement

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