TO FORMULATE AND EVALUATE SOLID LIPID NANOPARTICLES OF ITRACONAZOLE

Ankit Singh[1*], Mrs. Shivani Singh[1], Dr. Vandana Arora Sethi[1]

Abstract: The development of a solid lipid nanoparticle loaded with itraconazole was the aim of this investigation. The drug molecule's physiochemical and pharmacokinetic performance is constrained by the drug profile's poor solubility, toxicity, instability, incompatibility, and poor penetration. By using varied concentrations of different lipids and a hot homogenization procedure followed by an ultrasonication approach, itraconazole SLNs were created. The formulation's stability, drug content, in-vitro drug release, particle size analysis, scanning electron microscopy, and FTIR tests have all been assessed. Particle size analysis revealed that SLN prepared from the higher melting point lipid showed a larger particle size and with increased carbon chain length of fatty acids. %EE was 68.15 ± 25.65 %, and were in range of 42.50% (IF1) to 93.80% (IF12), drug content 96.99 ± 2.65 %. In-vitro release studies showed cumulative rate of release of 61.4 ± 10.7 % after 12 hours. All the formulations followed first order release kinetics. The FTIR investigation demonstrated that the medication was present in amorphous form and that there was no interaction between it and lipids. According to studies, greater lipid concentration, larger particle size, improved EE, and extended release are all beneficial. According to the developed formulation, the therapeutic activity of ITZ may be improved.

Keywords: Itraconazole, solid lipid nanoparticles, drug content, mean particle size

[1*] Lloyd Institute of Management and Technology, Plot No.-11, Knowledge Park-II, Greater Noida, Uttar Pradesh, India-201306

***Corresponding Author:** Ankit Singh
*****M. Pharm Research Scholar, Lloyd Institute of Management and Technology, Plot No.-11, Knowledge Park-II, Greater Noida, Uttar Pradesh, India-201306, Email-mailwithankit@gmail.com

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1. INTRODUCTION (SOLID LIPID NANO-PARTICLE)

A number of dosage forms, including aerosols, creams, capsules, injections, ointments, suppositories, liquids, pills, and more, have been used for years to treat both acute and chronic illnesses. There are now more options for drug delivery thanks to colloidal drug delivery systems such oil-in-water liposomes, micelles, emulsions, nanoparticles, and microparticles. (1)

These are colloidal solid particles with bound, entrapped, dissolved, or otherwise attached active principles. Nanoparticles have many advantages over conventional pharmaceutical delivery systems because of their small particle size, large surface area, and ability to change their surface properties. In general, nanoparticles can be used to target drug delivery, maintain drug action, boost bioavailability, solubilize drugs for intravascular transport, and improve drug stability against enzymatic degradation (2). Depending on the kind of inactive ingredient used, there are four different types of nanoparticles: polymeric nanoparticles (3), metal-based nanoparticles (4), biological nanoparticles (5), and lipid-based nanoparticles (6).

SLNs have been used as an alternative to liposomes, polymeric nanoparticles, microparticles, and emulsions as colloidal drug delivery techniques. They are composed of lipid particles with a diameter of a few nanometers. For regulated and targeted drug distribution, hydrophilic and lipophilic medications are added to SLNs. SLNs are composed of water, an emulsifier and/or co-emulsifier, and solid lipids. A typical solid lipid melts at temperatures above body temperature (37°C) when used in these delivery systems. Studies on lipids have included those on steroids, fatty acids, acylglycerols, triglycerides, waxes, and their combinations. All sorts of emulsifiers have been utilised, either singly or in combination, to stabilise the lipid dispersion. Among the nonionic emulsifiers include lecithin, bile salts such sodium taurocholate, and ethylene oxide/propylene oxide.

There are other emulsifiers that have been researched, including copolymers, fatty acid ethoxylates, sorbitan esters, and their combinations (7). Deionized water is used as the dispersion medium.

The medicine of interest, itraconazole, belongs to the class of antifungal agents with a lipophilic nature and is widely employed in research to treat a variety of fungal diseases. But the main point of contention centres on its adverse effects when given orally and parenterally, which, on the other hand, severely undermine its efficacy in managing antifungal medication. Based on the numerous clinical trials conducted, for example, oral capsule delivery is linked to hepatobiliary disorders including hyperbilirubinemia. Consequently, a multidisciplinary strategy has been used in the current study to create the solid lipid nanoparticles for topical distribution. Due to multiple reports from studies of its impacting affinity towards stratum corneum and subsequent importance in bioavailability, the goal behind the development of SLNs was to address this issue. The strategy is strengthened by defining the transition from oral to topical formulation and, additionally, by making precise changes to the functional components. Through research, it has been hypothesised that Itraconazole-loaded nanoparticles will function to reduce the negative effects in a healthier approach.

MATERIALS AND METHODS

Materials: Itraconazole (Yarrow Chem Pvt. Ltd. Mumbai), Stearic acid (Loba Chemie Pvt. Ltd), Compritol ATO888 (S.D. Fine Chemicals, New Delhi), Glyceryl monostearate (Central Drug House Pvt. LTD), Methanol (Central Drug House Pvt. LTD), Ethanol (Central Drug House Pvt. LTD), and Polyvinyl alcohol (Loba Chemie Pvt. Ltd) were purchased from the marketplace. All the other reagents and solvent used were of analytical grade.

Formulation of ITZ loaded SLN: The process of hot homogenization followed by ultrasonication is used to create SLNs. Three different types of lipids—stearic acid, GMS, and Compritol ATO 888—in varying lipid concentrations—1, 2, 5, and 10%—are used to make Itraconazole-loaded SLNs. For all formulations, the concentrations of the drug (10 mg) and surfactant (100 mg) remain unchanged. Polyvinyl alcohol, a surfactant, is employed as a stabilizer to improve the drug's solubility in lipids.

The preparation of ITZ-loaded SLNs involved heat homogenization method followed by ultrasonication method. Itraconazole and lipid were weighed and then dissolved in ethanol at various lipid concentrations, i.e.1, 2, 5, and 10%. A rotary flash evaporator may entirely extract organic solvents. Heating the lipids around 5-10 °Cover the lipid's melting point caused the implanted lipid layer to dissolve. Two different batch with 1.25% W/V and 2.5% W/V polyvinyl alcohol dissolved in distilled water to make 30 ml of preparation for each batch, which is then heated to the same temperature as the oil phase to create

the aqueous phase. After adding the hot aqueous phase to the oil phase, a mechanical stirrer is used to homogenise the mixture for 60 minutes (at 2000 rpm). The resultant coarse emulsion is agitated for 30 minutes at 400 rpm after being allowed to cool to room temperature. After that, it is ultrasonically processed for 10 minutes using a vibronic. Finally, amber-colored containers with itraconazole-loaded SLN were used for storage. (8-9)

Evaluation and characterization of ITZ loaded SLN

Entrapment Efficiency: The efficiency of entrapment of the itraconazole-loaded SLN is assessed by centrifugation. The nanoparticles are separated in a high-speed cooling centrifuge at 14,000 rpm for 90 minutes at 4 °C. The sediment is divided from the liquid supernatant. The supernatant solution is diluted to the right volume using a buffer. The amount of medication not contained in the SLNs might be measured using the UV-spectrophotometer. To determine their proportion of EE, the absorbance at 345 nm is examined.
(9) The entrapment efficiency is calculated with the help of the following formula.

% Entrapment efficiency (EE)= Amount taken - Free drug / Amount taken x 100.

Particle size analysis:
Using a laser light scattering apparatus (LS545; COULTERS) at a fixed angle of 90° and
25 °C, photon correlation spectroscopy (PCS) was used to measure the mean diameter of SLNs in the dispersion. The volume distribution was used to analyse the results of the particle size study. Distilled water is used to dilute 1 ml of sample before to testing. (10)

Morphology:
Scanning electron microscopy is used to assess the optimal formulation's average particle size and surface shape. The material was made to spread out on an aluminium stub and let to air dry. Air dried sample was subsequently coated with gold by using a Hitachi Ion-Sputter E-1010 for 40 seconds. The Hitachi S- 3400 Scanning Electron Microscope was used to take the photos. (11)

In Vitro Drug Release:
The dialysis bag method was used to assess the in-vitro release of itraconazole from itraconazole-loaded SLN formulations using 0.5% SLS solution as the dissolving medium. We employed a dialysis membrane with a pore size of 2.4nm and a molecular weight cutoff of 14,000. Prior to use, the membrane was soaked in distilled water for 15 minutes. The dialysis bag was filled with 1 mg of drug equivalent in SLN dispersion, which was stored in a beaker with 250 ml of 0.5%SLS solution in it as a buffer.At a constant temperature of 37± 2 °C, the dissolving media was constantly agitated at 100 rpm. At regular intervals, samples were taken out, and the equivalent volume of fresh dissolving medium was added in their place. By measuring the absorbance at 345nm against an appropriate solvent blank, samples were spectrophotometrically examined for the presence of the drug itraconazole. Each experiment was carried out three times, and the average results were recorded. (12, 13)

Kinetic Modeling: The results of the in vitro drug release investigation of nanoparticles were fitted with severalkinetic equations, including Higuchi's model (cumulative% drug release vs. square root of time), first order (log% drug remaining vs time), and zero order (cumulative% release vs. time)". For the linear curve produced by regression analysis of the aforementioned plots, r2 and k values were computed. The Korse• Meyer's Peppas model determines the precise method via which the SLN formulations follow (log drug release vs log time). (14)

FT-IR Studies:
Using an IR spectrophotometer, the drug's interaction with lipids is investigated. FTIR research on drugs, lipids, and their physical combinations were conducted. Using a Shimadzu FT-IR spectrophotometer, the FT-IR spectra of a pure drug and a combination of a drug and an excipient is obtained. The resolution is 4 cm-1, and the scanning range is 450-4000 cm-1. KBr pellets are used to prepare samples. (15)

Stability Studies:
All Itraconazole-loaded SLN formulations were subjected to stability investigations at 25 2
°C and 60± 5 RH for 1 month, as well as at 4 °C, in accordance with modified ICH recommendations, and the entrapment efficiency was determined for all formulations at 2-week intervals for the same duration. (16)

2. RESULT AND DISCUSSION

Physico-chemical Properties: Obtained SLN dispersion was fluid in character, white in colour, and odourless. Even after centrifugation, it remained stable and exhibited no signs of sedimentation (2000 rpm for 30 minutes).

Entrapment Efficiency: The figure 1 displayed

the outcomes of EE. The following is a discussion of the outcome of lipid and surfactant concentration.

Compritol > Glyceryl Monostearate > Stearic Acid

Compritol demonstrated the highest EE among the 3 lipids when compared to stearic acid and glyceryl monostearate. This may be because the existence of long chain fatty alcohols may result in the formation of a less organised solid lipid matrix, which leaves room for medication molecules to fit.

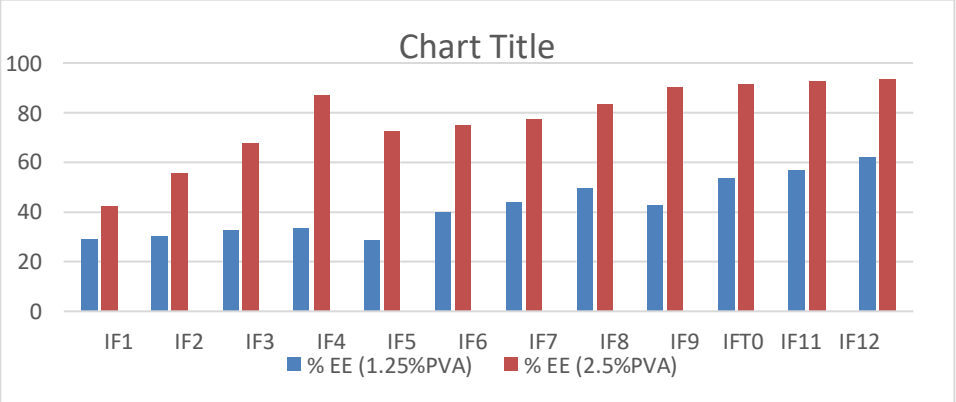

Figure 1: Comparison of EE of PVA 1.25 and 2.5%

Particle Size Analysis: The dynamic light scattering approach was used to estimate the particle size analysis of the SLN formulations. Table 1 displays the particle size values. The relationship between increased lipid content and larger particle size suggests that emulsifying effectiveness has decreased and particle agglomeration has increased. The three lipids are arranged in decreasing order of particle size as follows:

Glyceryl monostearate< stearic acid< compritol.

Based on the findings, an SLN formulation in the nanometric size range was obtained by optimising the 5% concentration of the three lipids.

Table 1: Comparison of Particle Size

S. No.	Conc. %	Mean Particle Size
1	1 % Stearic acid	434 nm
2	2 % Stearic acid	358 nm
3	5 % Stearic acid	56 nm
4	10 % Stearic acid	238 nm
5	1 % Glyceryl monostearate	634 nm
6	2 % Glyceryl monostearate	463 nm
7	5 % Glyceryl monostearate	84 nm
8	10 % Glyceryl monostearate	398 nm
9	1% Compritol ATO 888	745 nm
10	2 % Compritol ATO 888	532 nm
11	5 % Compritol ATO 888	109 nm
12	10 % Compritol ATO 888	475 nm

Scanning Electron Microscopy (SEM) Studies: By using a scanning electron microscope, the morphology of an itraconazole-loaded SLN dispersion was investigated.

For SEM investigations, the optimum formulation, IF11 (a formulation containing 5% compritol), was chosen. The SEM image was displayed in figure 2. It became clear from the SLN dispersion that the particles were under 500 nm in size, spherical in form, and nearlysmooth on the surface.

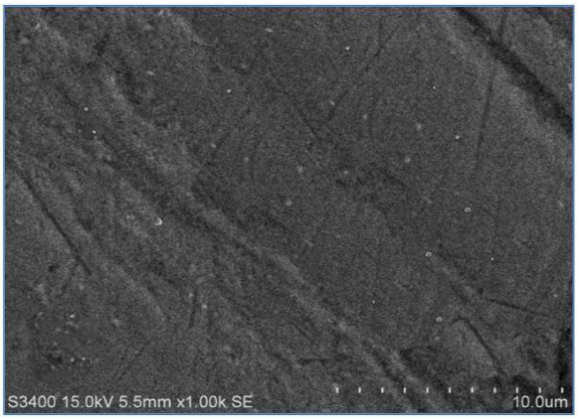

Figure 2: SEM image of formulation IF11

In Vitro Release Studies:

Similar biphasic drug release patterns were seen in SLN containing three different lipids, with a burst release occurring within 30 minutes and a persistent release thereafter. The medication attached to the nanoparticles' surface may be the cause of the burst release.

The findings indicated that the release was mostly influenced by the lipid concentration, i.e., a rise in lipid concentration causes a decrease in release rate.

Due to its larger carbon chain length compared to the other two lipids, compritol demonstrated more sustained release than stearic acid and glyceryl monostearate. The order of drug release from the three lipids as follows:

Compritol > Glyceryl monostearate > Stearic acid

According to the evaluated findings, release rate is decreased by greater lipid concentration. The type of fatty acids had a big impact on the release, and the longer their carbon chains, the slower the drug released.

Release Kinetics:

Release kinetics was used to study the kinetics and mechanism of drug release. The valuesfor n, k, and r2 are shown in figure 1.3. First-order release was present in all formulations, and this release exhibited stronger linearity than the zero-order or Higuchi models.

The Korsemeyer's-Peppas model was used to pinpoint the precise mechanism of the release kinetics. Results showed that non-Fickian release kinetics were observed in all SLN formulations.

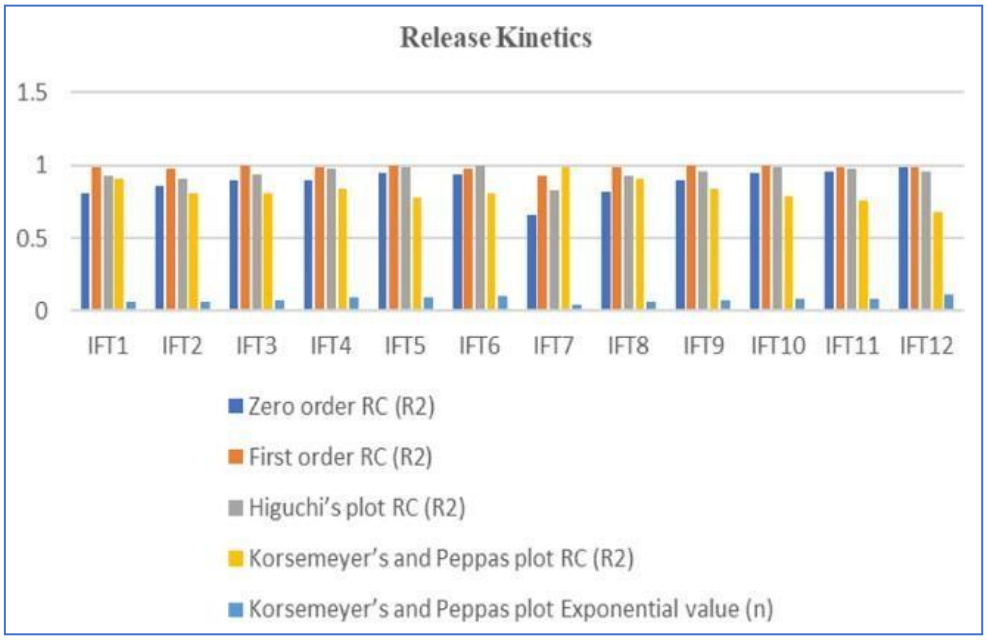

Figure 3: Release Kinetics

FT-IR Studies:

To check the compatibility of the drug (itraconazole) and the lipids (stearic acid, glyceryl monostearate, and compritol) utilised, FT-IR tests were conducted. The FTIR spectra of the drug in its pure form, the lipids used, and its physical mixture were assessed. In figure 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9, the FTIR spectra are displayed.

"Itraconazole was a pure drug that exhibits bond vibrations at the following frequencies: 3396 cm^{-1} for COOH stretching, 3057 cm^{-1} for aromatic C-H stretching, 2925 cm^{-1} for aliphatic C-H stretching, 1710 cm^{-1} for C• ostretching, 1610 cm^{-1} for C-C stretching, 1594 cm^{-1} for C-N stretching, 1497 cm^{-1} for aliphatic C-H bending, 1132 cm^{-1} for C•O stretching".

"Stearic acid's FTIR spectrum reveals absorption bands of C-H stretching at 2957 cm^{-1}, C-H bending at 1465 cm^{-1}, O-H bending at 1433 cm^{-1}, and C-C stetching at 1099 cm^{-1}; glyceryl monostearate revealed absorption bands of C-H stretching at 3015 cm^{-1}, C-H bending at 1735 cm-1, and 0 - H bending at 1290 cm^{-1}."

Overall, however, observations showed no alteration in the colour, appearance, lumpiness, or condition of eitherdrug, excipient alone, or both together. By doing an FTIR analysis of the drug and excipients and comparing the peaks of the mixture with those of the drug and individual excipients, the compatibility was confirmed.

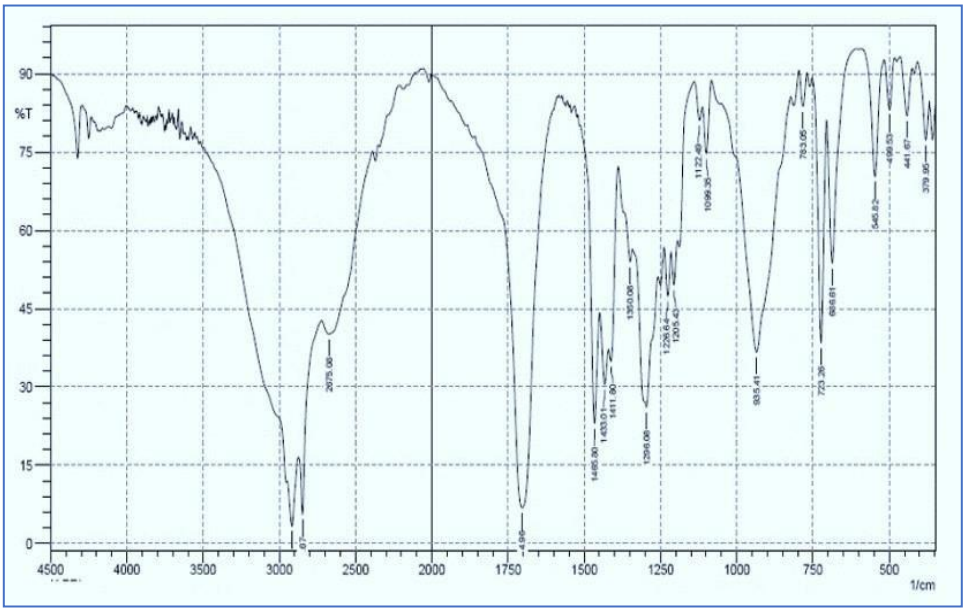

Figure 4: FTIR Spectra of Stearic Acid

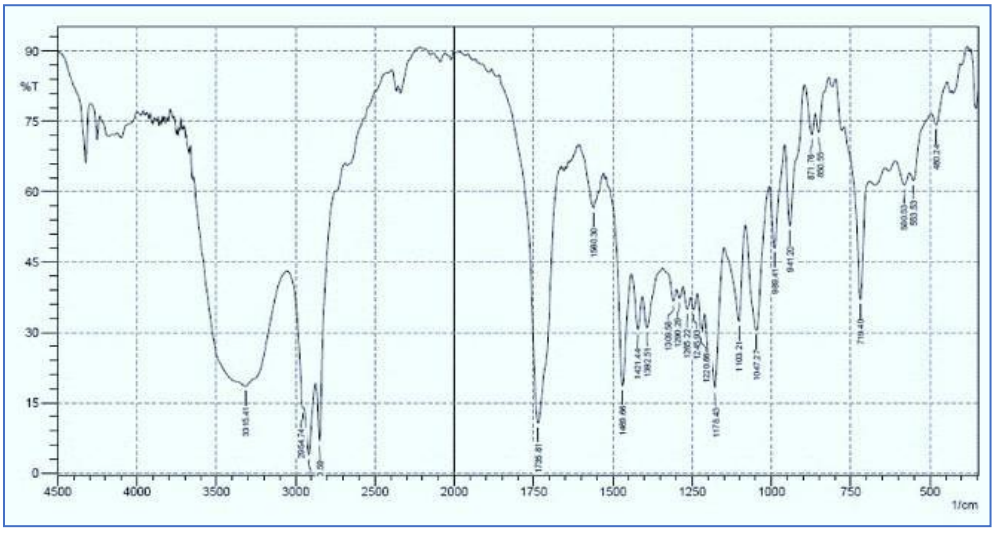

Figure 5: FTIR Spectra of GMS

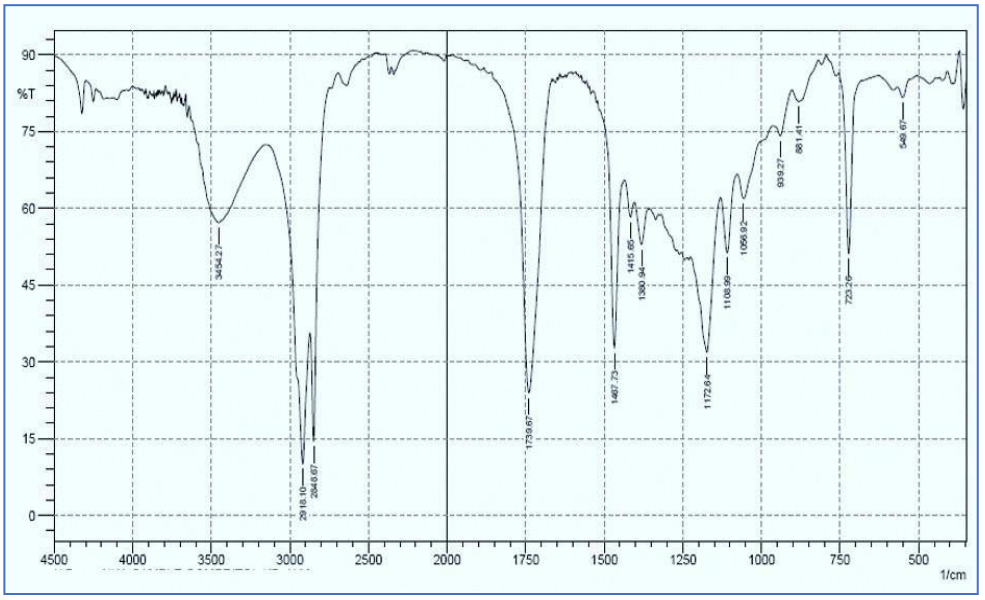

Figure 1.6: FTIR Spectra of Compritol

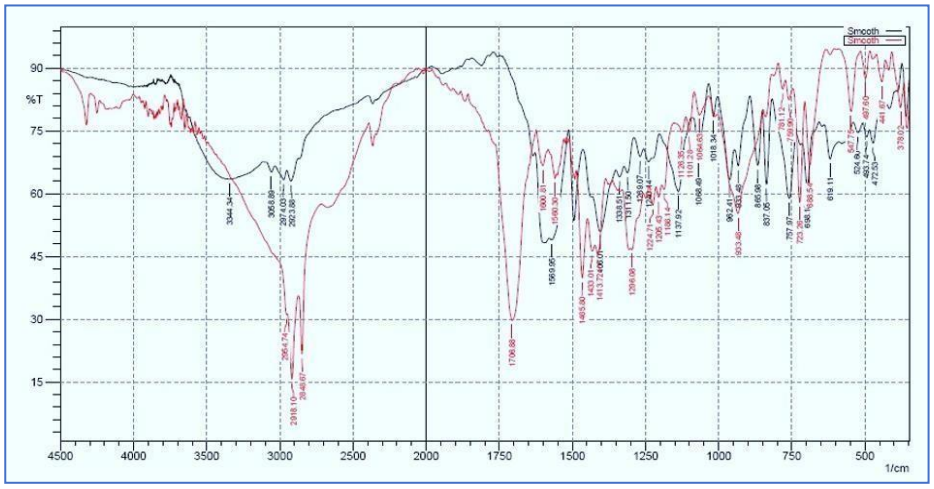

Figure 1.7: FTIR Spectra of ITZ+Stearic acid

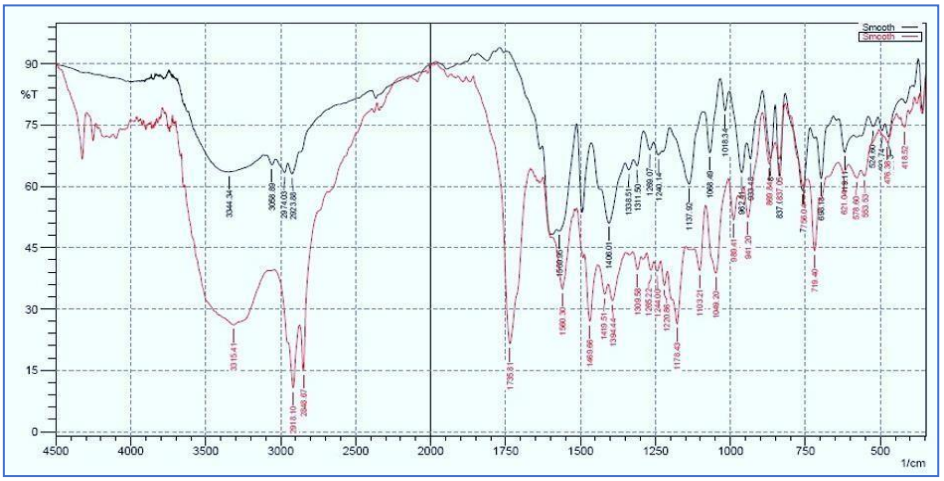

Figure 8: FTIR Spectra of ITZ+GMS

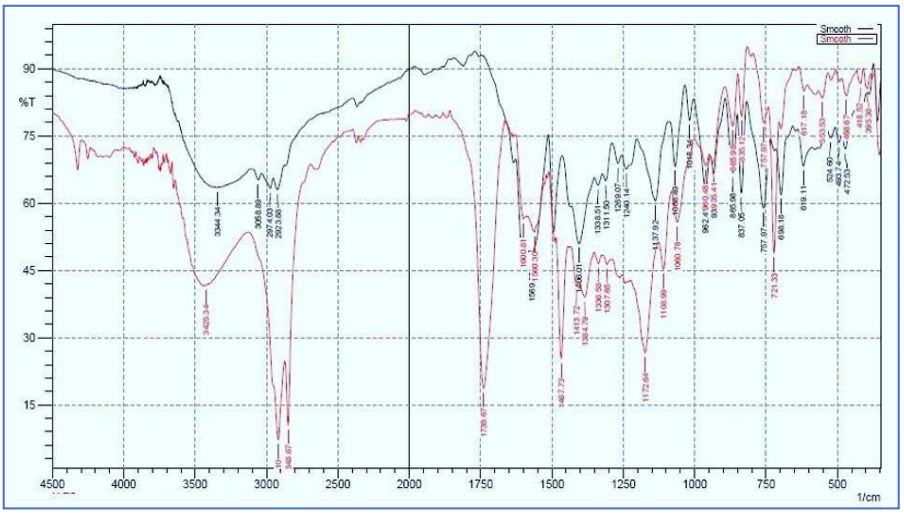

Figure 9: FTIR Spectra of ITZ+Compritol

STABILITY STUDIES:

Two sets of SLN formulations were examined for stability studies. "One set were stored at refrigeration temperature (4 ± 2°C) and the other set were stored at 25 ± 2 °C and 60 % ± 5%RH at the stability chamber for 1 month and the entrapment efficiency were determined at 2-week intervals". According to the findings, decreased entrapment efficiency was seen during storage; this could be because drugs were ejected during lipid modification. Thus, it was determined that the formulations showed no appreciable change in entrapment efficiency at 4 °C and 25 °C. Refer table 3.

Table 3: Stability Study

Formulation	% EE at 0 days	% EE at 4 °C		% EE at 25 °C	
		15 Days	30 Days	15 Days	30 Days
IF1	42.50 ±0.19	42.00 ±0.15	41.50 ±0.16	41.00 ±0.21	40.50 ±0.17
IF2	55.67±0.16	55.10 ±0.18	54.60 ±0.17	54.20 ±0.18	54.10 ±0.21
IF3	67.76±0.15	67.40 ±0.17	67.00 ±0.19	66.70 ±0.18	66.20 ±0.2
IF4	87.08±0.21	86.90 ±0.17	86.60 ±0.18	86.30 ±0.19	85.00 ±0.13
IF5	72.50±0.18	72.10 ±0.20	72.00 ±0.13	71.70 ±0.14	71.30 ±0.16
IF6	75.10±0.22	74.80 ±0.19	74.30 ±0.15	74.30 ±0.13	74.10 ±0.14
IF7	77.76±0.12	77.50 ±0.15	77.30 ±0.17	77.20 ±0.16	76.10 ±0.21
IF8	83.60±0.16	83.10 ±0.14	82.50 ±0.19	81.40 ±0.15	81.30 ±0.12
IF9	90.60±0.21	90.00 ±0.17	89.20 ±0.14	89.00 ±0.19	88.10 ±0.11
IF10	91.58±0.15	91.20 ±0.21	91.00 ±0.17	90.90 ±0.18	90.60 ±0.13
IF11	92.9±0.23	92.9 ±0.19	92.85 ±0.15	92.3 ±0.18	92.1 ±0.16
IF12	93.80±0.21	93.8 ±0.11	93.7 ±0.15	93.10 ±0.20	92.90 ±0.13

3. CONCLUSION

The present research study was to estimate the effect of lipids on the preparation of SLNs of itraconazole. Research result suggests that the hot homogenization process followed by ultrasonication method was a viable method for preparing SLNs of itraconazole. Finally, it was determined that a workable approach is hot homogenization followed by ultrasonication. The SLNs' particle sizes demonstrated that the SLNs made from higher melting point lipid had larger particle sizes and longer fatty acid carbon chains. Studies using FTIR indicated that there is no drug-lipid interaction. According to studies, a rise in lipid concentration led to larger particles, increased EE, and maintained the drug's release. Since Compritol ATO 888 had a greater EE and prolonged drug release profile than GMS and stearic acid, it was selected as the optimum lipid for SLN formulation.

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