4. Preformulation Study

4.1. Introduction

The term "Pre-formulation" refers to a collection of studies that are carried out before formulation development. These investigations demonstrate that a dosage form with predetermined properties can be successfully developed on a scientifically supported basis. Pre-formulation studies can save a lot of money and time, which further reduces the difficulties associated with formulation development. The physico-chemical characteristics of the APIs and their compatibility studies with the excipients were performed and presented in this chapter (1).

4.2. Materials and Instruments

Table 4.1 List of Materials:

Materials & Reagents	Manufacturers
Luliconazole	Sun Pharmaceutical Industries Ltd., Vadodara
Tavaborole	Symed Labs Limited, Hyderabad
Capmul MCM C8	Abitec Corporation, USA
Cremophore EL	Sigma Aldrich, India
Pluronic F68	Sigma Aldrich, India
Coconut oil	Marico India Pvt. Ltd., Mumbai
Softemul AS	Mohini Lab, Mumbai
Softemul AE	Mohini Lab, Mumbai

Table 4.2 List of Equipments

Equipment/Instrument	**Manufacturer/Supplier**
Digital Analytical Balance	Shimadzu, Japan
Differential Scanning Calorimetry (DSC)	Shimadzu, Japan
Magnetic Stirrer	Remi equipment Pvt. Ltd., India
Vortex mixer	Spinix, Japan
Ultraturrax T25	IKA, Mumbai
FT-IR spectrophotometer	Shimadzu 8400S, Japan
Centrifuge	Remi Instrument, India
Bath Sonicator	Remi equipment Pvt. Ltd., India

4.3 Methodology

4.3.1 Organoleptic Properties

Visual characteristics of Tavaborole were studied on the basis of Color and appearance.

4.3.2 Melting Point determination by Glass Capillary Method

The capillary tube with the sealed end was filled with a small amount of the drugs. The melting point equipment was used to record the temperature at which the drug melted after being introduced within the capillary tube. By comparing the practically acquired melting point value with the standard reported value of pharmaceuticals, the identification of the drug was confirmed (2).

4.3.3 Infrared Spectroscopy

The IR-spectrum of the Luliconazole and Tavaborole was evaluated in the solid state by potassium bromide (KBr) pellet method. KBr (previously dried in oven) was mixed with approximately 1-2 % of drug sample taken in a mortar and ground into a fine powder using a pestle. In a motorized pellet press, the KBr pellets were made by applying 10- to 12-metric-ton of pressure. Then, using an FTIR spectrometer (Bruker, USA), a spectrum of the pellets was acquired by scanning them across a wavelength range of 4000-400 cm-1 (3).

4.3.4 Differential scanning Calorimetry (DSC)

To examine the thermal behaviour of a medicament, differential scanning calorimetry (DSC) was carried out using a DSC-41 (Shimadzu, Japan). A drug sample (2–3 mg) was obtained and placed in an aluminium pan under pressure from the outside. In order to produce an inert environment and prevent oxidation due to oxygen, this aluminium pan was heated from 25°C to 300°C at a scanning rate of 10°C/min under nitrogen flow rates of 40 ml/min (4).

4.3.5 Solubility study for selection of oil

Solubility of Luliconazole and Tavaborole was found out in various oils and surfactants on the basis of saturation solubility. 1 ml of the solvent was taken and incremental amounts of drug were added until the solvent was saturated and no more drug dissolve. This mixture was centrifuged at 3000 rpm to separate the undissolved drug and the supernatant was collected and analyzed using suitable analytical method as described in chapter 3 (section 3.3.1 for Luliconazole and 3.4 for Tavaborole) after suitable dilution as required (5-7). Screening of oil was carried out for preparation of nanoemulsion by solubility study, the oil which had highest solubilization capacity was selected.

4.3.6 Determining the Optimal Surfactant and Cosurfactant:

A variety of surfactants, cosurfactants, coconut oil, and capmul MCM C8 were utilized to produce nanoemulsions. The stability of these nanoemulsions was monitored over a 15-day period, noting any instances of cracking, creaming, or phase separation. The choice of surfactant was based on these observations (8-9).

4.3.7 Primary screening of solid lipids and surfactants:

Solubility study of Luliconazole and Tavaborole in different solid lipids and surfactants:

The lipid solubility of Luliconazole and Tavaborole was assessed using different lipids such as compritol ATO 888, precirol ATO 5, dynasan 118, softemul AS, softemul SE, glyceryl tristearate, and monegyl GN05. Surfactants like span 40 and span 60 was also evaluated. To test solid lipids, each drug was introduced to 100 mg of the lipid, heated to 70°C in a test tube. Solubility was visually inspected to ensure complete dissolution. Additional drug quantities were added in molten lipid incrementally until saturation occurred. The solid lipids with the highest solubilization capacity for both drugs were selected for further use (10).

4.3.8 Selection of the ratio of solid lipid to liquid lipid:

To determine the optimal lipid blend for Luliconazole and Tavaborole, the chosen solid and liquid lipids with the highest solubilizing potential were combined in varying weight-to-weight ratios (50:50, 60:40, 70:30, and 80:20). The mixtures were stirred at 100 rpm for 30 minutes at 70°C. After cooling to ambient temperature, they were evaluated for lipid miscibility. This was done by applying a thin layer of the lipid mixture onto filter paper and visually inspecting for oil droplets, which would indicate lipid separation. The ideal lipid combination was identified as one with a melting point exceeding 40°C and no evidence of oil droplet formation on the filter paper, making it suitable for NLC formulation (11).

4.4 Result and Discussion:

4.4.1 Organoleptic characterization of the drug:

Table 4.3: Organoleptic characterization of the drug

Sr. No.	API	Properties	Observation	Standard
1	Luliconazole	Appearance	Light yellow Crystalline Powder	Light yellow crystalline Powder (1)
		Odour	Odourless	Odourless (1)

2	Tavaborole	Appearance	White Powder	White Powder (2)
		Odour	Odourless	Odourless (2)

The Organoleptic characteristics are matching with the reported characteristics of the compounds. This indicates that the samples are authentic.

4.4.2 Determination of Melting Point:

Table 4.4: Observed Melting Point

Method	API	Observed Melting Point (°C)	Reported Melting Point (°C)
Glass Capillary Method	Luliconazole	152	150-153 (1)
	Tavaborole	120	118-120 (2)

Observed melting points of Luliconazole and Tavaborole were close to the reported melting point range. From this, it is evident that the samples were authentic.

4.4.3 Fourier Transfer Infrared Spectroscopy (FT-IR) of Drug

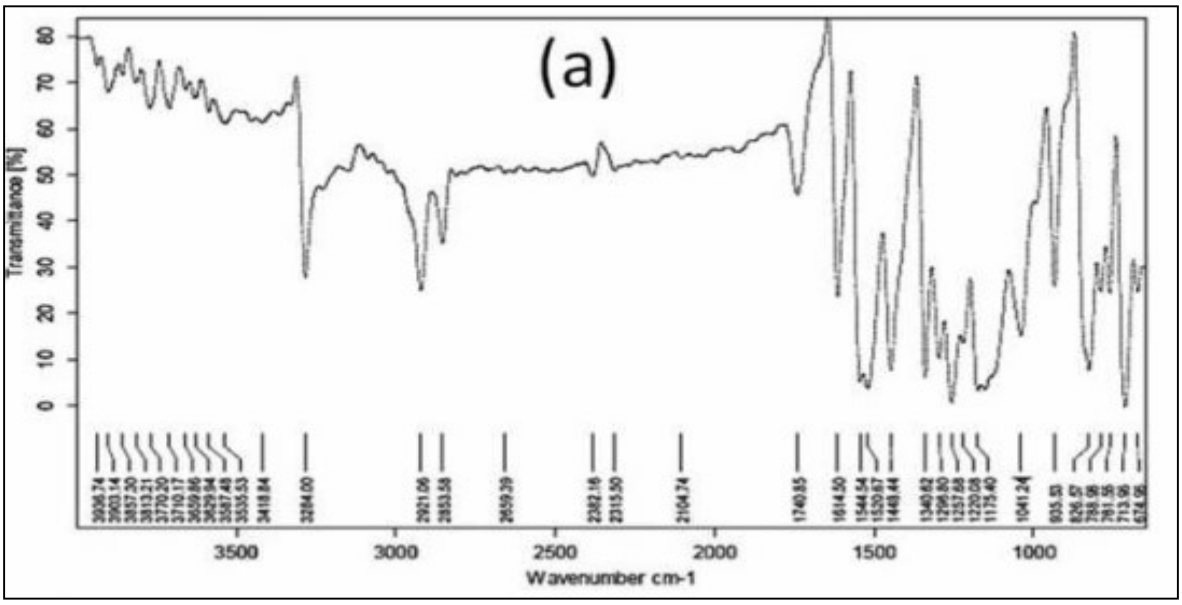

Figure 4.1 Reference spectrum of Luliconazole (3)

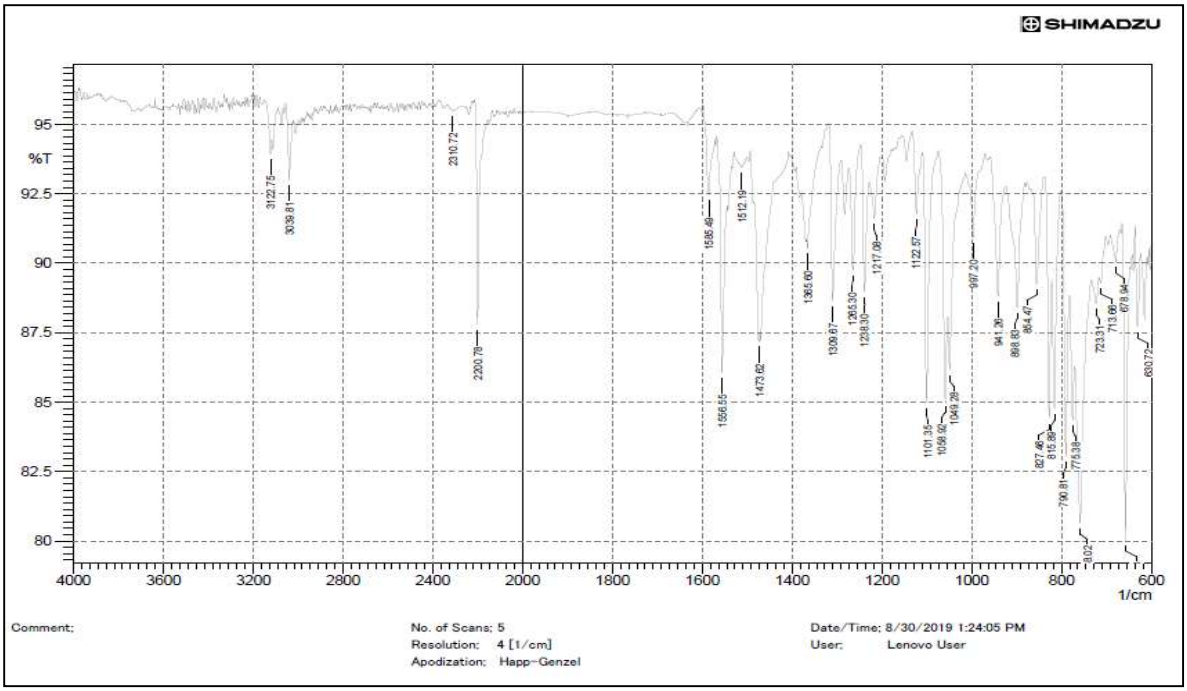

Figure 4.2: FTIR spectrum of Luliconazole

Table 4.5: Interpretation of FTIR of Luliconazole

Type of Vibration	Standard	Observed	Inference
C-H Aromatic stretch	3000-3100	3122.75 3039.81	Present
C-H aliphatic stretch	2840-3000	2941	Present
S-H stretching	2550-2600	2527 2614	Present
C- N Stretch	2222-2260	2310 2200	Present
C=C aromatic stretch	1450-1650	1512 1556	Present
Aromatic C=C For Chlorobenzene	1446,1478,1584	1473 1585	Present
C-Cl stretch	550-850 1089-1096	678	Present

In table 4.5, the comparison between observed and standard peaks showed authenticity of Luliconazole.

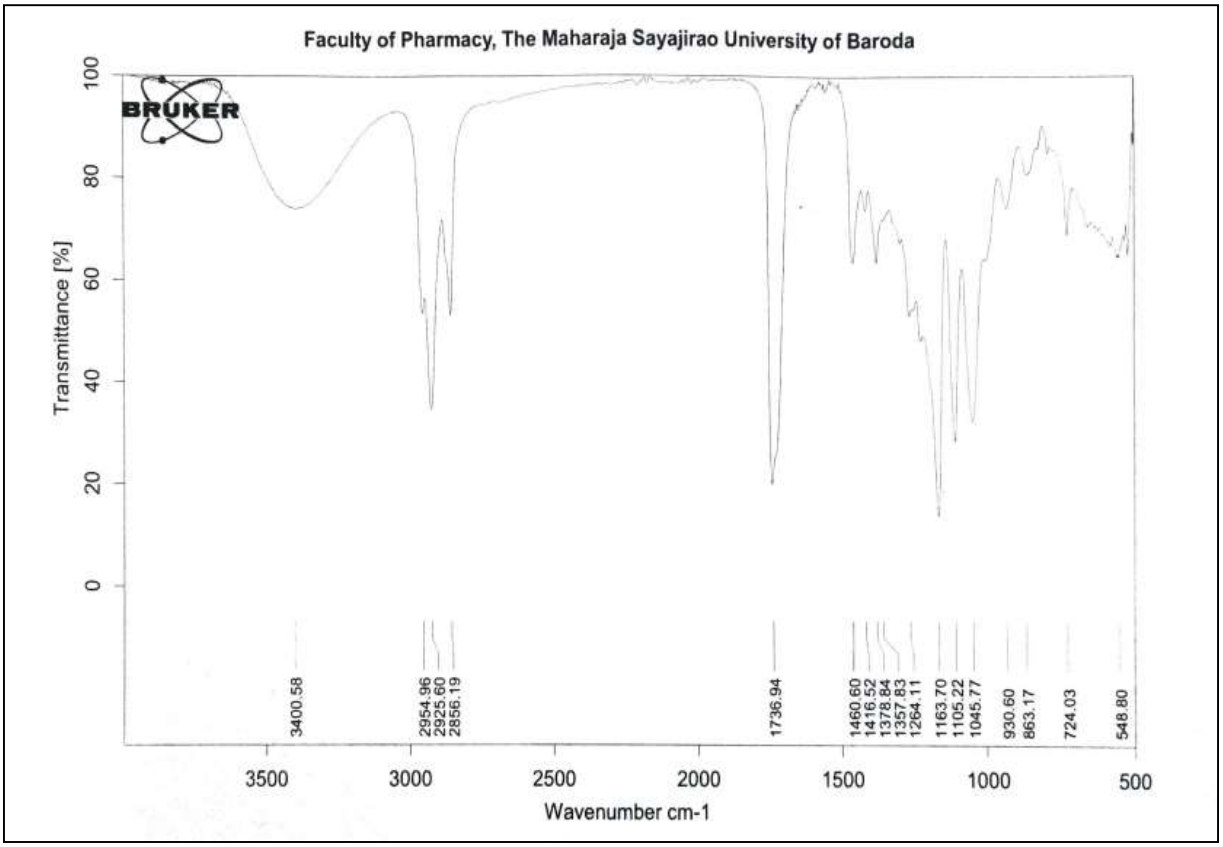

Figure 4.3: FTIR spectrum of physical mixture of Luliconazole and Capmul MCM C8

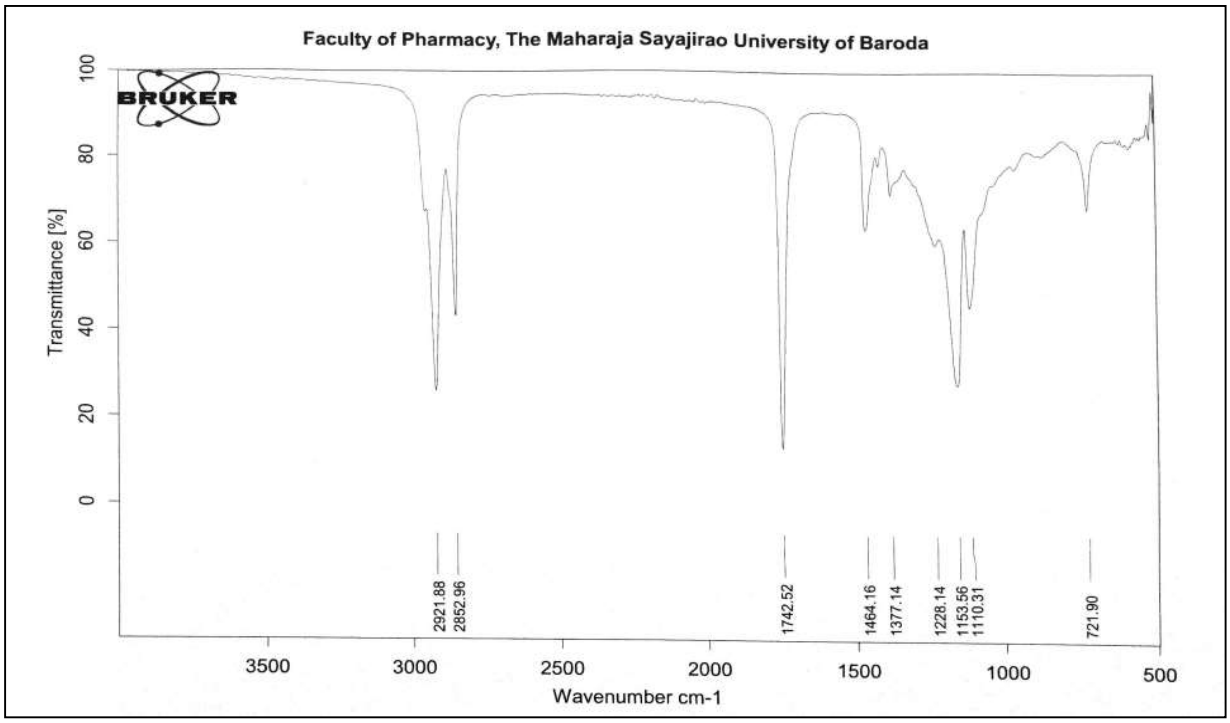

Figure 4.4: FTIR spectrum of physical mixture of Luliconazole and Coconut oil

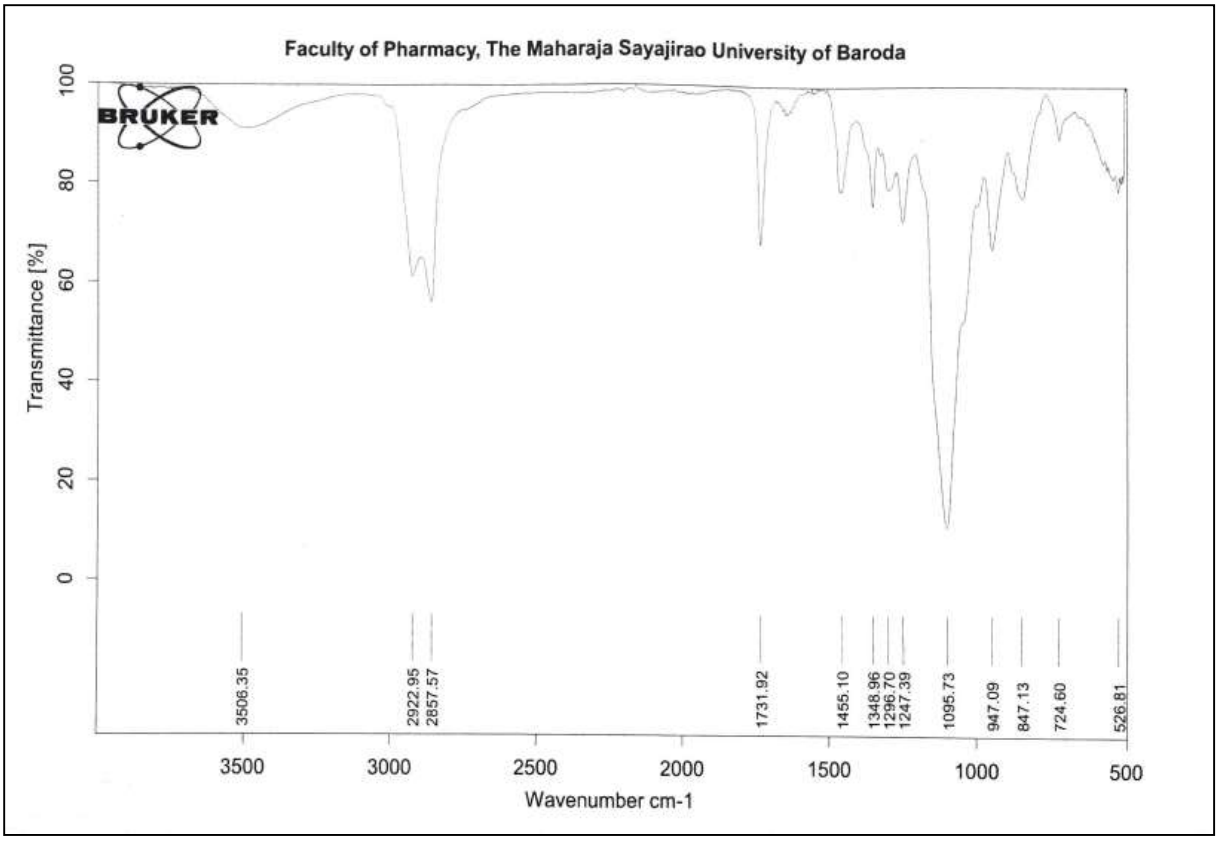

Figure 4.5: FTIR spectrum of physical mixture of Luliconazole and Cremophore EL

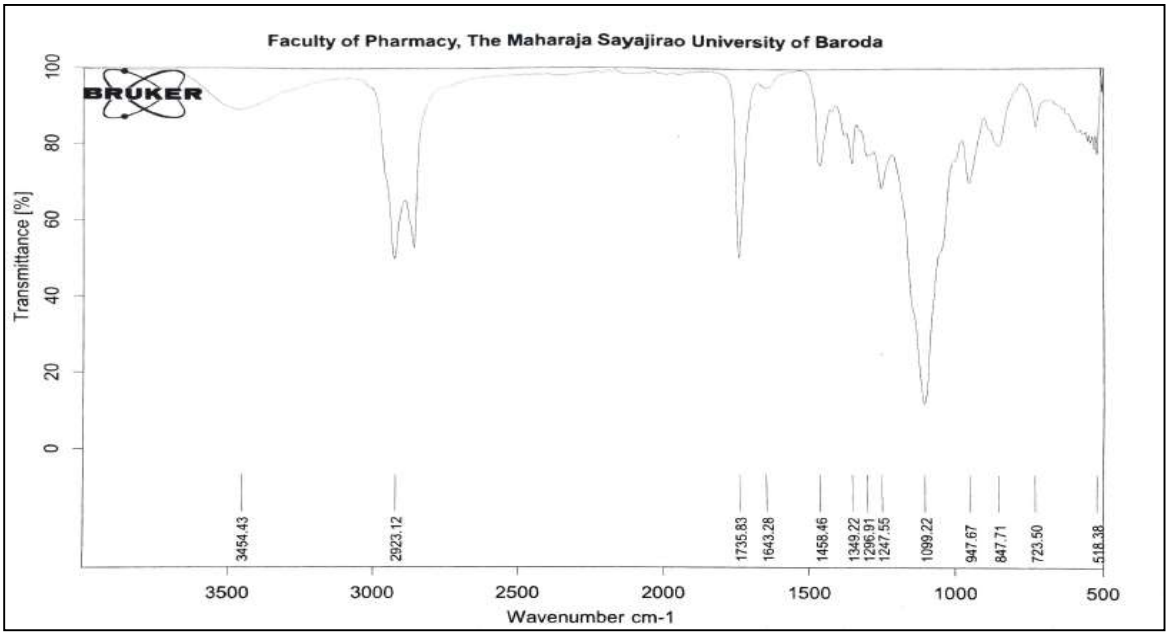

Figure 4.6: FTIR spectrum of Luliconazole + Physical mixture of Capmul MCM C8, Coconut oil, Cremophore EL, Pluronic F127 (For Nanoemulsion)

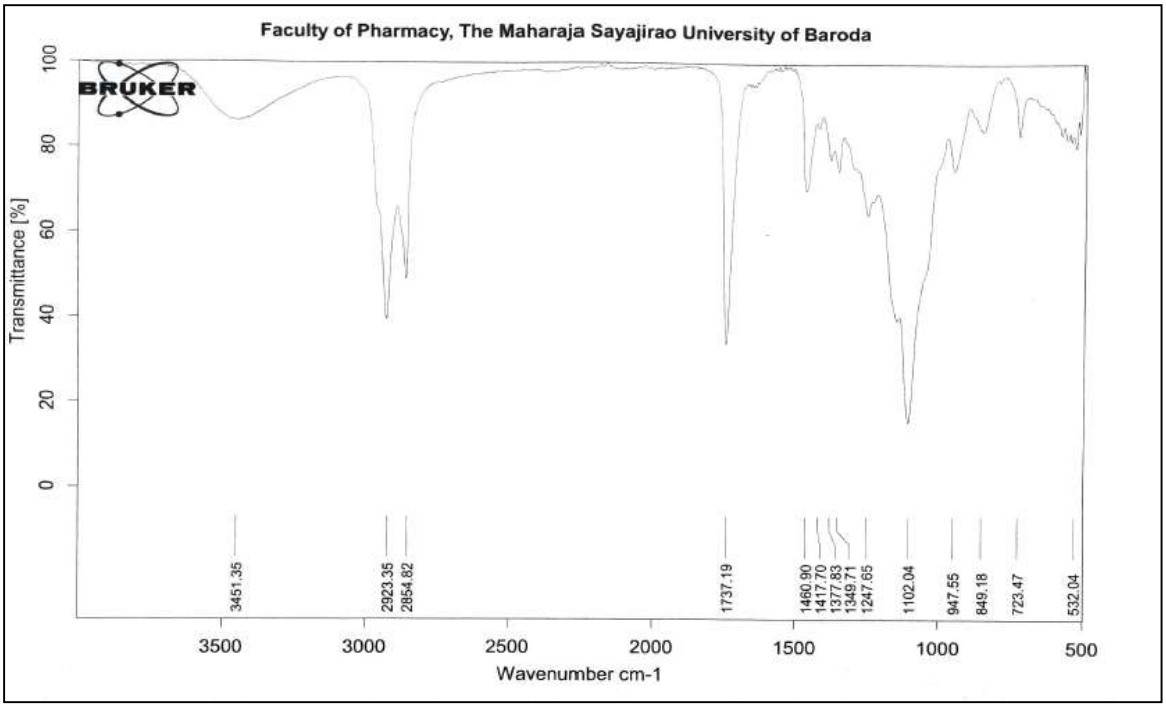

Figure 4.7: FTIR spectrum of Luliconazole + Physical mixture of Capmul MCM C8, Softemul AS, Cremophore EL, Pluronic F127 (For NLCs)

Presence of all characteristic peaks in above FTIR spectrums (figures 4.3-4.7) indicates the excipients compatibility between Luliconazole and the physical mixtures of Luliconazole with excipients.

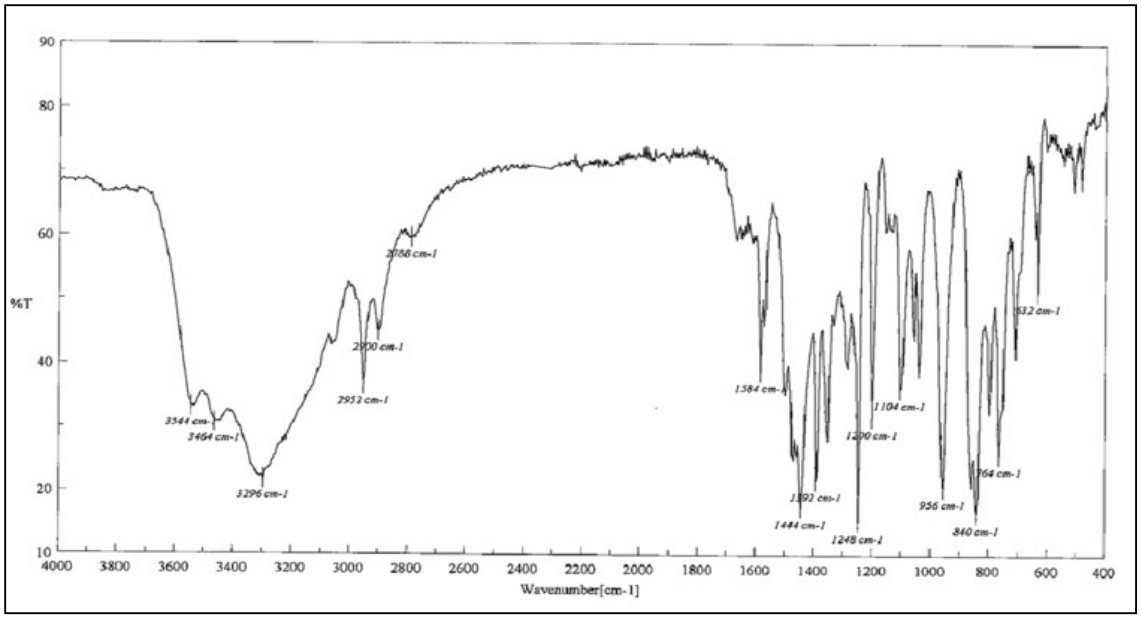

Figure 4.8 Reference FTIR spectrum of Tavaborole (4)

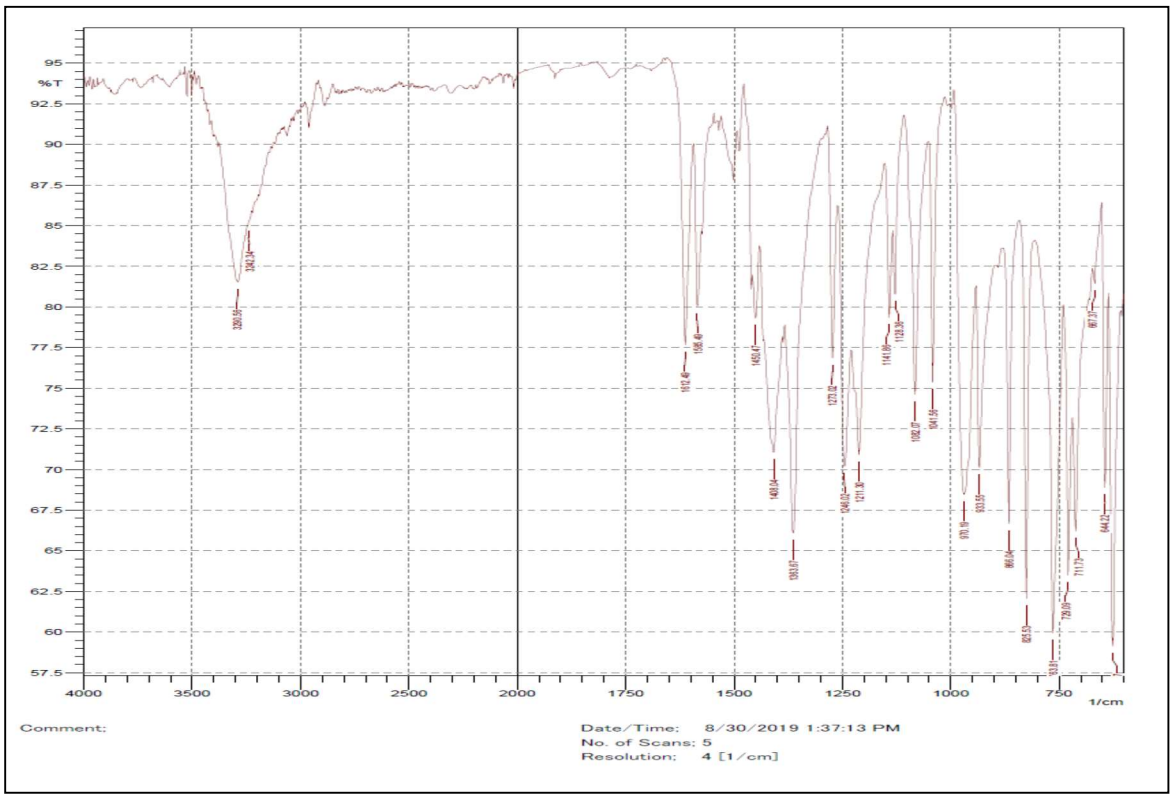

Figure 4.9: FTIR spectrum of Tavaborole

Table 4.6: FTIR Interpretation of Tavaborole

Type of Vibration	Standard	Observed	Inference
O-H stretching	3550-3200	3242.34	Present
C=C Stretching	1650-1600	1612.49	Present
B-O stretching	1340-1370	1363.67	Present
C-F stretching	1400-1000	1246.02	Present
C- O Stretching (Aliphatic ether)	1150-1085	1082.07	Present

In table 4.6, the comparison between observed and standard peaks showed authenticity of Tavaborole.

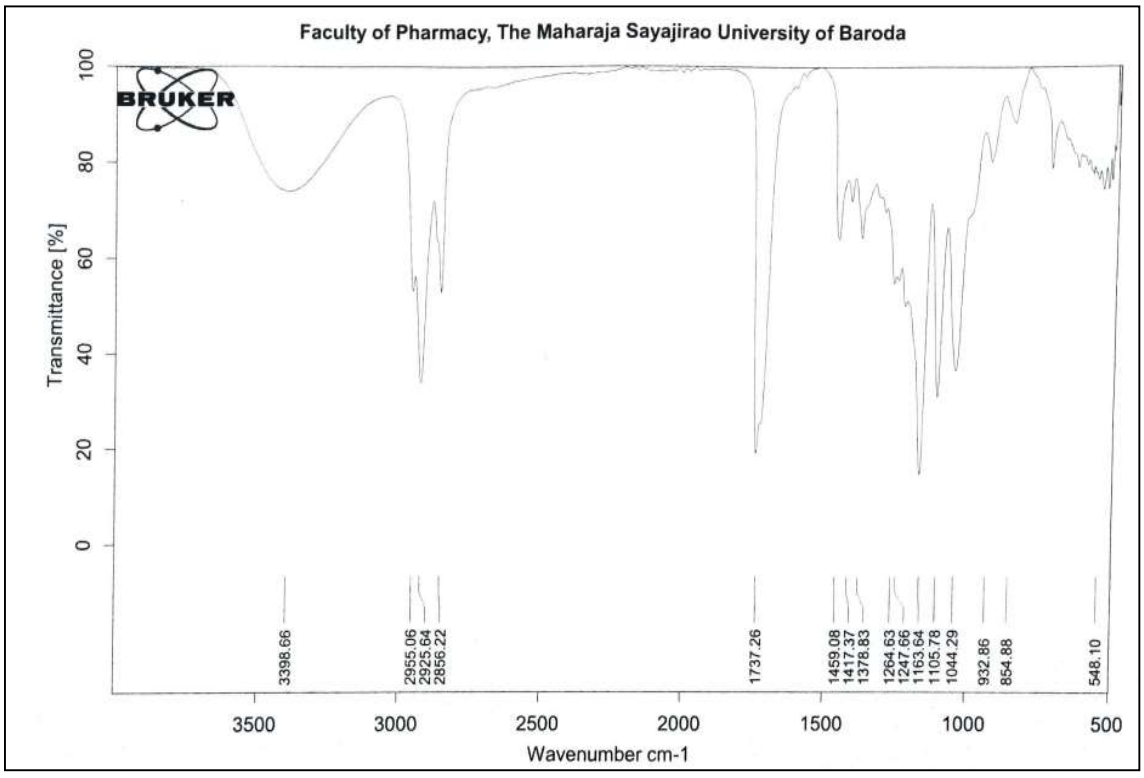

Figure 4.10: FTIR spectrum of physical mixture of Tavaborole and Capmul MCM C8

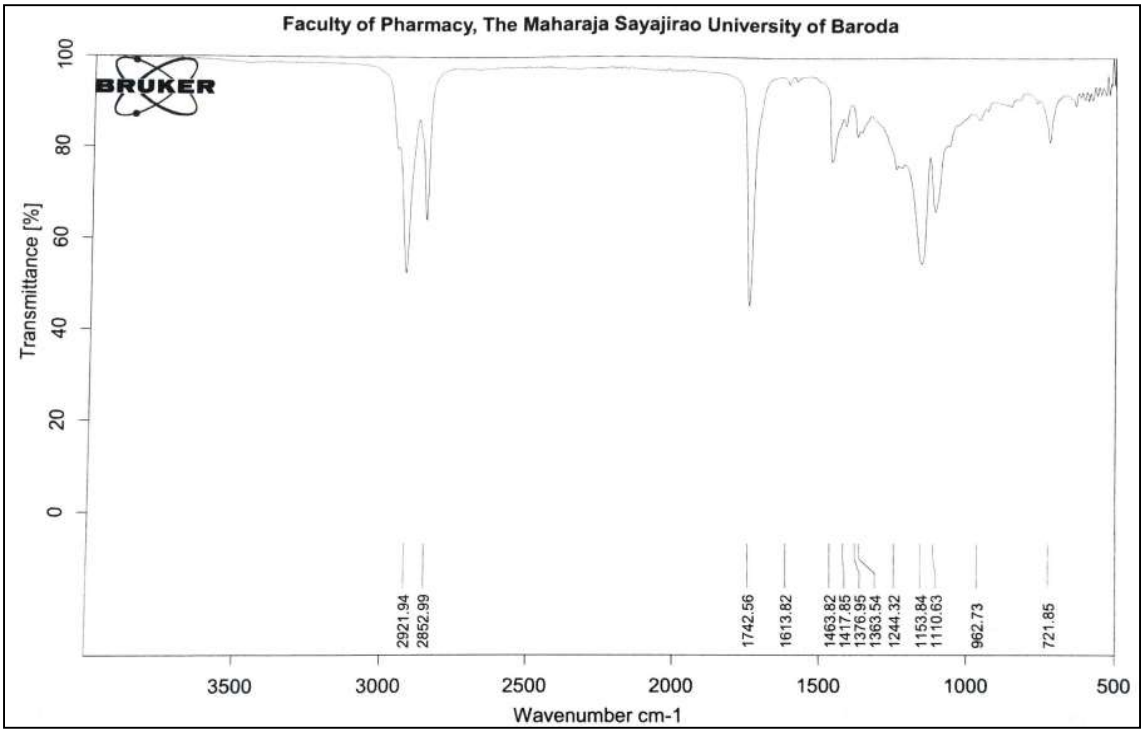

Figure 4.11: FTIR spectrum of physical mixture of Tavaborole and Coconut oil

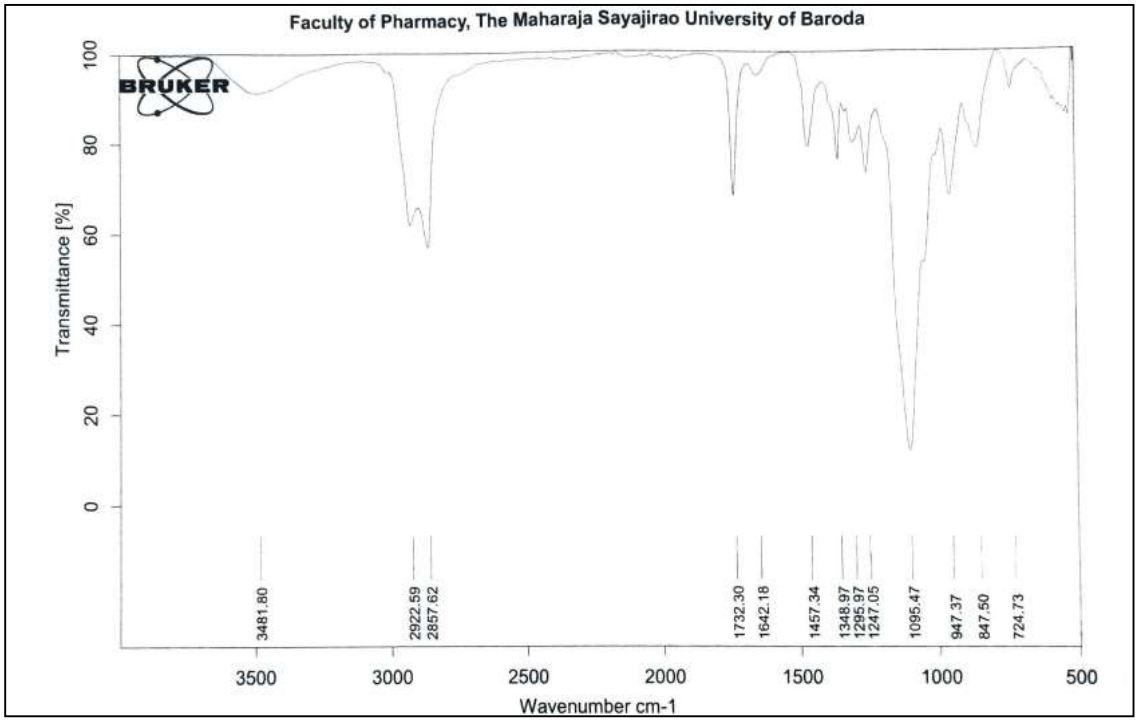

Figure 4.12: FTIR spectrum of physical mixture of Tavaborole and Cremophore EL

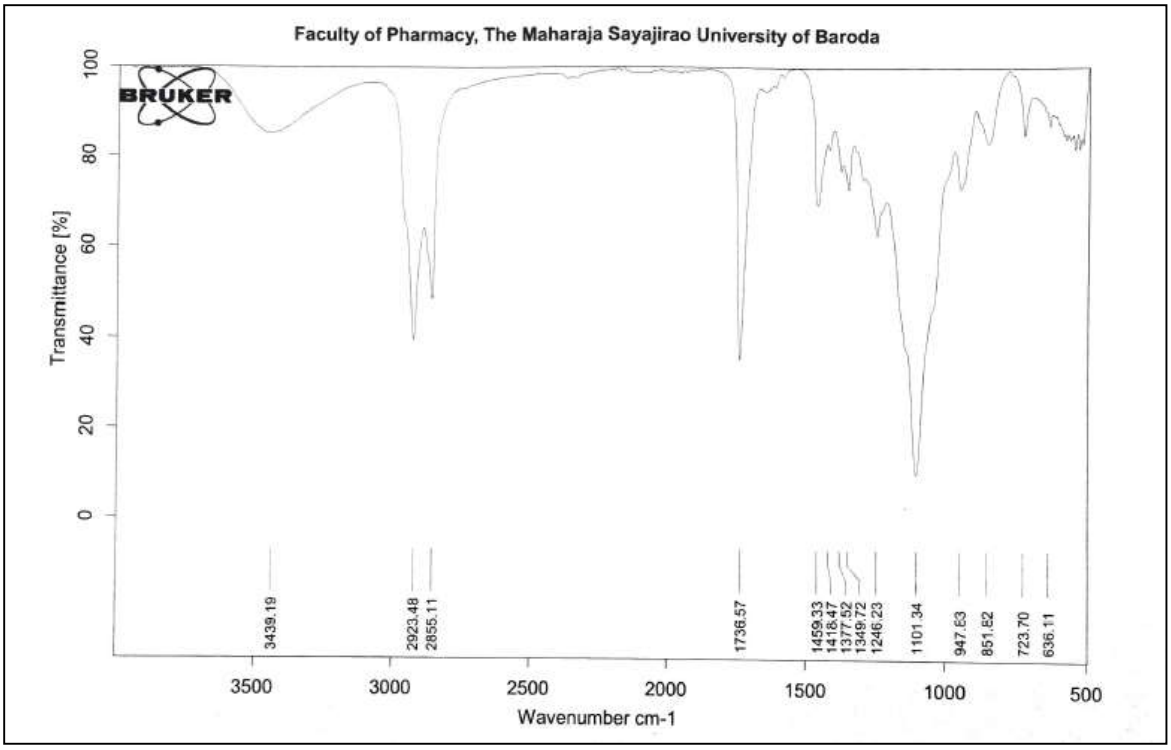

Figure 4.13: FTIR spectrum of Tavaborole + Physical mixture of Capmul MCM C8, Coconut oil, Cremophore EL, Pluronic F127 (For Nanoemulsion)

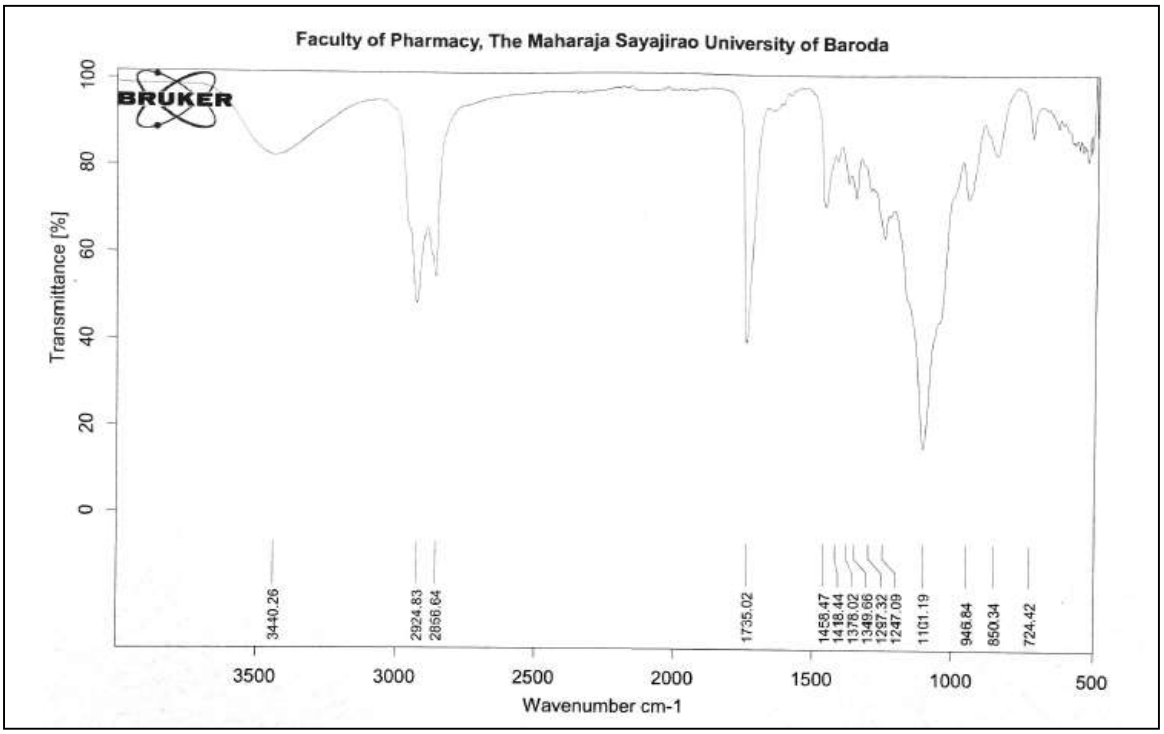

Figure 4.14: FTIR spectrum of Tavaborole + Physical mixture of Capmul MCM C8, Softemul SE, Cremophore EL, Pluronic F127 (For NLCs)

Presence of all characteristic peaks in FTIR spectrums (figure 4.10-4.14) indicates the excipients compatibility between Tavaborole and the physical mixtures of Tavaborole with the excipients.

4.4.4 Differential scanning Calorimetry (DSC):

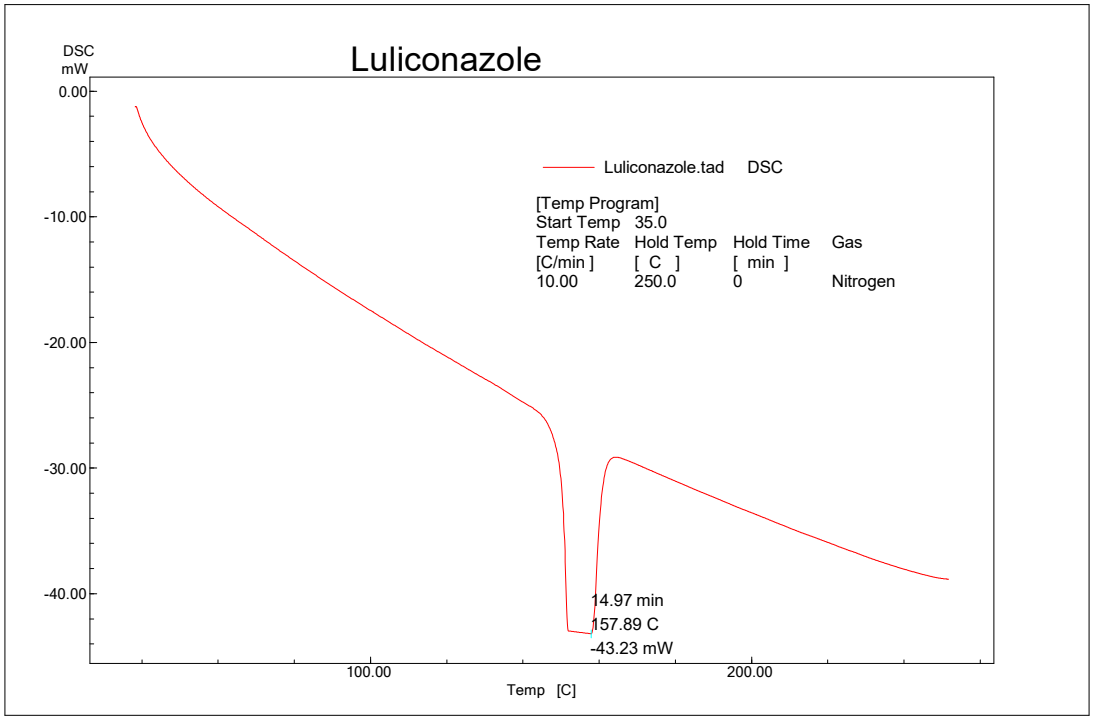

Figure 4.15: DSC thermogram of Luliconazole

The reported melting point of Luliconazole is 152 °C and from the DSC graph, a sharp phase transition (melting point) was obtained at 157.89 °C which was almost same as the reported melting point of Luliconazole (1).

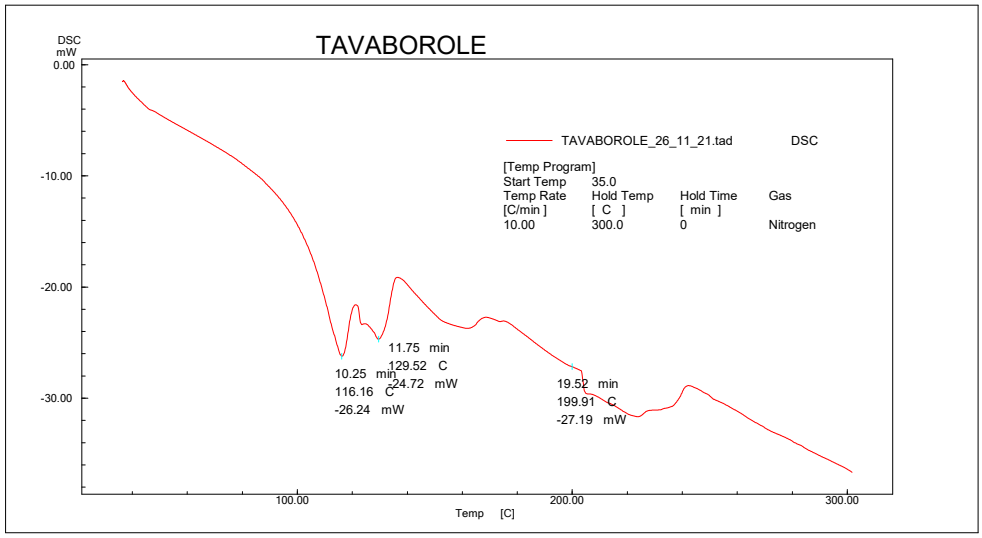

Figure 4.16: DSC thermogram of Tavaborole

The reported melting point of Tavaborole is 120 °C and from the DSC graph, a sharp phase transition (melting point) was obtained at 116.16 °C which was almost same as the reported melting point of Tavaborole (2).

4.4.5 Solubility study for selection of oil and surfactants

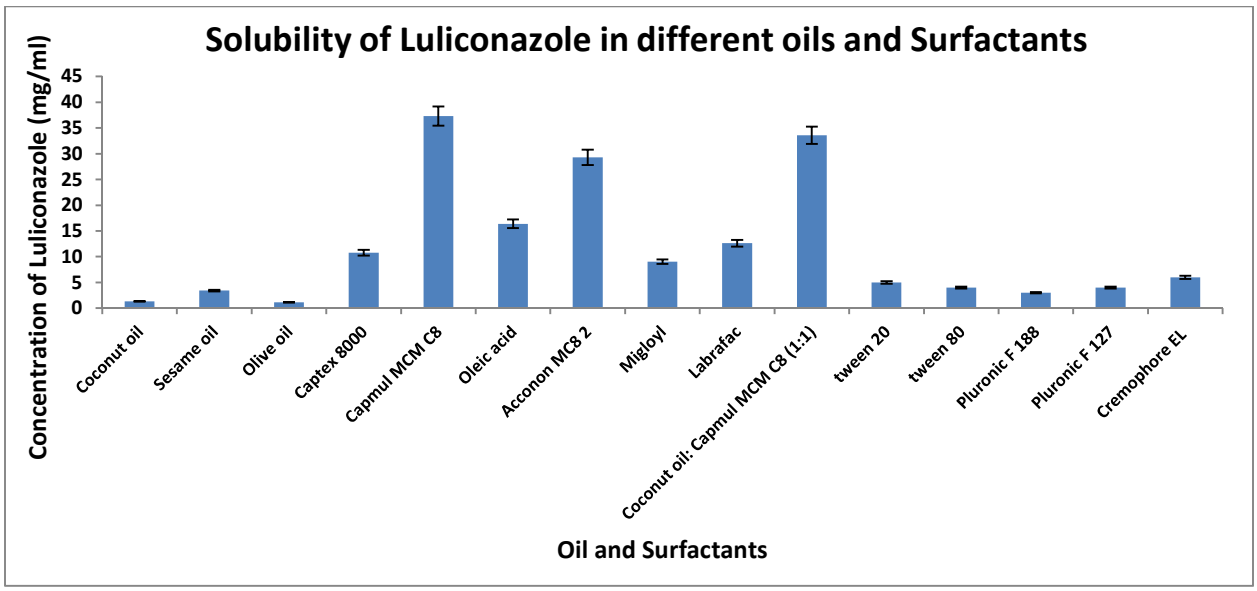

Figure 4.17: Solubility of Luliconazole in different oils

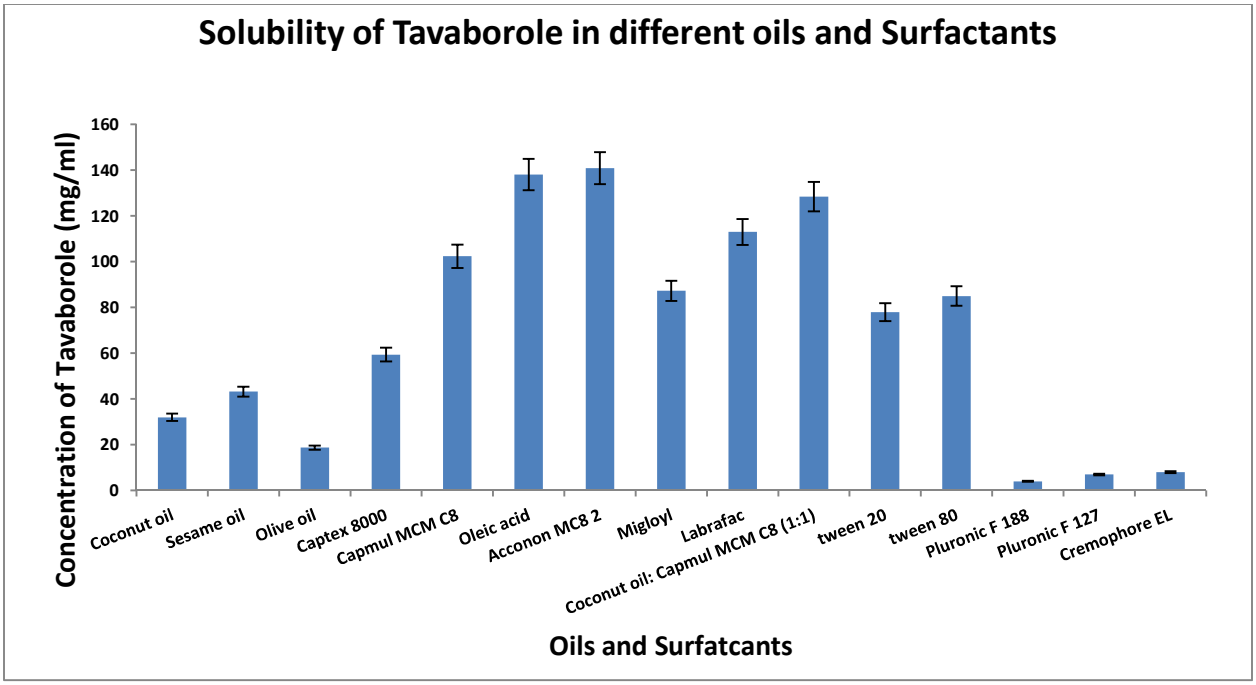

Figure 4.18: Solubility of Tavaborole in different oils, liquid lipids and surfactants

The highest solubility was found in Capmul MCM C8 so it was selected oil phase. It was reported that coconut oil shows the antifungal activity (12) therefore; to enhance the antifungal activity of formulations, combination of Capmul MCM C8 and coconut oil was selected as oil phase for Luliconazole nanoemulsion. For Tavaborole, Acconon MC8-2 showed highest solubility. However, nanoemulsions prepared by using Acconon MC8-2 were not stable. Therefore, Tavaborole nanoemulsions were formulated by using Capmul MCM C8 and coconut oil as oil phase.

4.4.6 Screening of the Surfactant and Cosurfactant:

The selection process for the surfactant and cosurfactant involved testing various combinations, with a fixed oil phase comprising a mixture of coconut oil and capmul MCM C8 in a 70:30 ratio.

Table 4.7: Screening of Surfactant and Cosurfactant

Batch No.	Surfactant	Cosurfactant	Concentration of Surfactant (%W/W)	Concentration of cosurfactant (%W/W)	Visual observation
1	Tween 20	-	1	-	Phase separation
2	Tween 20	-	2	-	Phase separation

3	Tween 80	-	1	-	Phase separation
4	Tween 80	-	2	-	Phase separation
5	Cremophore EL	-	1	-	1st Day: Milky and Stable 2nd Day to 6th Day: Separate but redispersible 7th Day: Phase separation
6	Cremophore EL	-	2	-	1st Day to 7th Day: Milky and Stable 7th Day to 14th Day: Separate but redispersible 15th Day: Phase separation
7	Cremophore EL	Pluronic F127	2	1	1st Day to 15th Day: Milky and Stable
8	Cremophore EL	Pluronic F127	2	2	1st Day to 15th Day: Milky and Stable
9	Cremophore EL	Transcutol	2	1	1st Day : Milky and Stable 2nd Day: Phase separation
10	Cremophore EL	Transcutol	2	2	1st Day : Milky and Stable 2nd Day: Phase separation
11	Cremophore	PEG 400	2	1	1st Day : Phase

	EL				separation
12	Cremophore EL	PEG 400	2	2	1st Day : Phase separation

The selection of the surfactant and co-surfactant was based on the emulsion's stability over 15 days. The data from this optimization study, as shown in the table 4.7, led to the choice of Cremophor EL as the surfactant and Pluronic F 127 as the co-surfactant. The HLB (hydrophilic lipophilic balance) of the surfactant and cosurfactant is very important factor to indicate its ability to stabilize emulsion. For the stability of nanoemulsion RHLB value of oil phase is very important. When the HLB value of the emulsification system is close to that of the oil, the surfactant molecules arrange more closely on the oil-water interface film. This leads to a stronger interfacial film and smaller emulsion droplets, which improves emulsion stability. Cremophore EL efficiently forms micelles, which enhances the drug encapsulation. It is quite effective at solubilising very hydrophobic drugs. The mechanism by which it entraps the lipophilic drugs is through micellar solubilisation (13).

4.4.7 Solubility study for selection of lipid

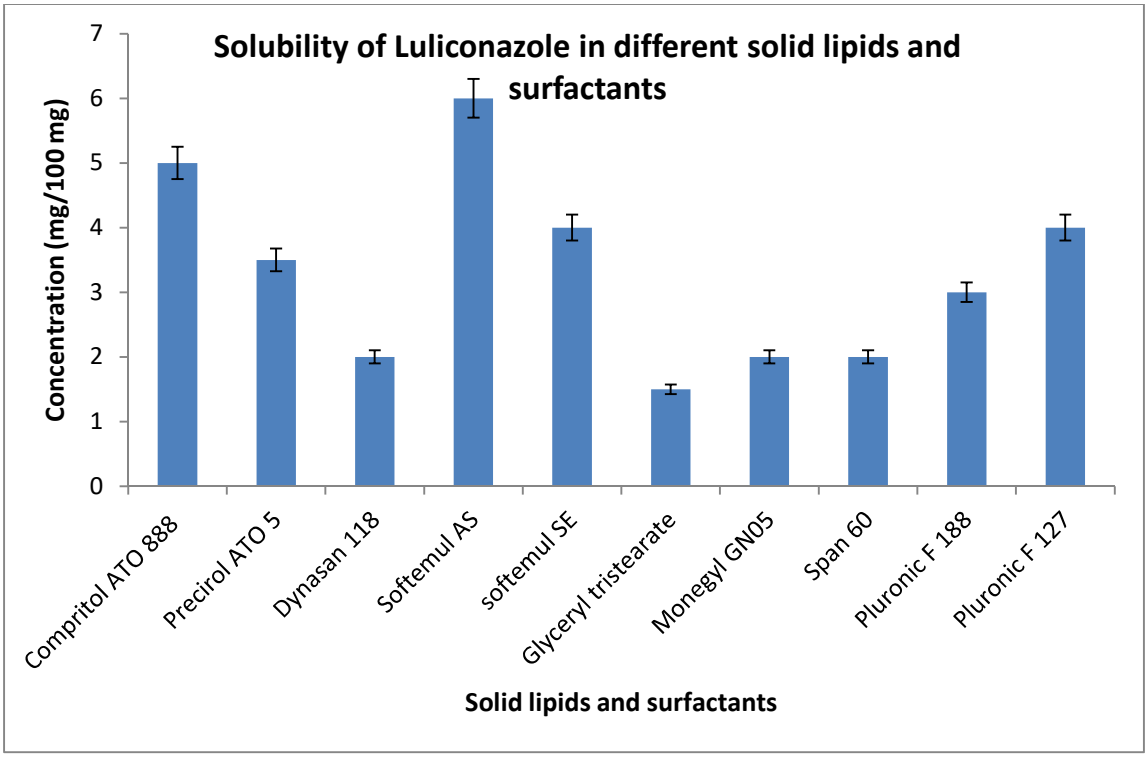

Figure 4.19: Solubility of Luliconazole in different solid lipids and surfactants

From the figure 4.19 it was found that Softemul AS and Pluronic F 127 showed highest solubility for Luliconazole so selected as liquid lipid and surfactant respectively. Capmul MCM C8 was selected as liquid lipid based on solubility.

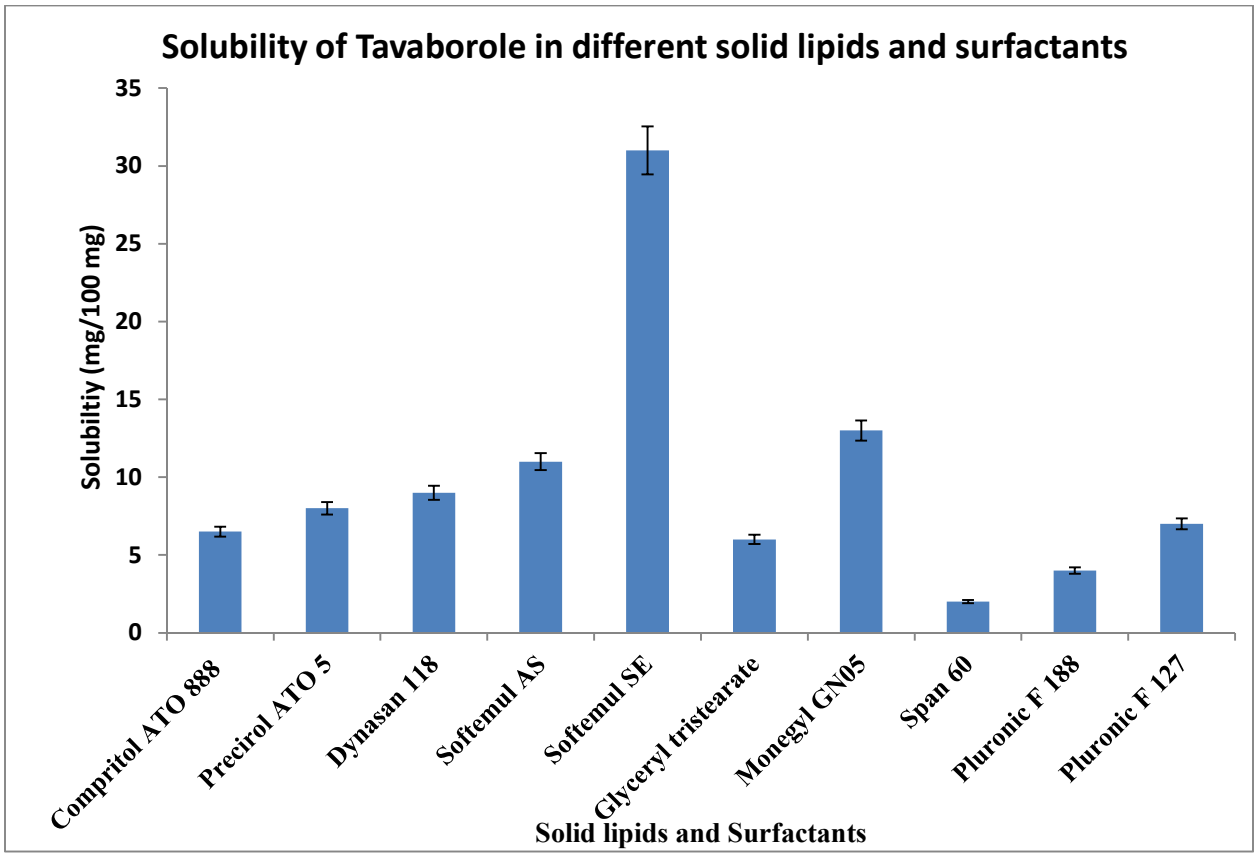

Figure 4.20: Solubility of Tavaborole in different solid lipids and surfactants

From the figure 4.20 it was found that Softemul SE and Pluronic F 127 showed highest solubility for Tavaborole so selected as liquid lipid and surfactant respectively. Capmul MCM C8 was selected as liquid lipid based on solubility.

4.4.8 Selection of the ratio of solid lipid to liquid lipid:

Visual observation of samples smeared on the filter paper indicated that binary lipid in the ratio 60:40 w/w (solid: liquid lipid ratio) was optimum for formulation of the NLCs.

4.5 References:

1. Khanna, Deepshikha, and Subhash Bharti. "Luliconazole for the treatment of fungal infections: an evidence-based review." *Core evidence* (2014): 113-124.
2. Gupta, Aditya K., and Sarah G. Versteeg. "Tavaborole–a treatment for onychomycosis of the toenails." *Expert Review of Clinical Pharmacology* 9.9 (2016): 1145-1152.
3. Mishra, Shubham & Gupta, Revathi. (2022). Formulation and Evaluation of Niosomal Gel of Antifungal Luliconazole. *Journal of Drug Delivery and Therapeutics*. 12. 47-54. 10.22270/jddt.v12i6-S.5705.
4. Luliński, Sergiusz, et al. "A tautomeric equilibrium between functionalized 2-formylphenylboronic acids and corresponding 1, 3-dihydro-1, 3-dihydroxybenzo [c][2, 1] oxaboroles." *New Journal of Chemistry* 31.1 (2007): 144-154.
5. Chaurasia, Gita. "A review on pharmaceutical preformulation studies in formulation and development of new drug molecules." *Int J Pharm Sci Res* 7.6 (2016): 2313-2320.
6. Kala, Shivani, and Divya Juyal. "Preformulation and characterization studies of aceclofenac active ingredient." *The Pharma Innovation* 5.9, Part B (2016): 110.
7. Knopp, Matthias Manne, et al. "Comparative study of different methods for the prediction of drug–polymer solubility." *Molecular pharmaceutics* 12.9 (2015): 3408-3419.
8. Alves-Silva, I., et al. "Preformulation studies of itraconazole associated with benznidazole and pharmaceutical excipients." *Thermochimica Acta* 575 (2014): 29-33.
9. Tambe, Srushti, et al. "Recent advances in amorphous solid dispersions: preformulation, formulation strategies, technological advancements and characterization." *Pharmaceutics* 14.10 (2022): 2203.
10. Kovačević, Anđelka B., Rainer H. Müller, and Cornelia M. Keck. "Formulation development of lipid nanoparticles: Improved lipid screening and development of tacrolimus loaded nanostructured lipid carriers (NLC)." *International Journal of Pharmaceutics* 576 (2020): 118918.

11. Pardeike, Jana, et al. "Development of an itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application." *International journal of pharmaceutics* 419.1-2 (2011): 329-338.
12. Wang, Qiuxia, et al. "Study on the relationships between the oil HLB value and emulsion stabilization." *RSC advances* 13.35 (2023): 24692-24698.
13. Gelderblom, Hans, et al. "Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation." *European journal of cancer* 37.13 (2001): 1590-1598.