4. Preformulation Study

4.1. Introduction

The term “Pre-formulation” owing to its name refers to a group of studies which are conducted prior to formulation development. These studies prove to be the scientifically driven foundation for successful development of a robust dosage form with pre-defined characteristics. By performing pre-formulation studies, loads of money and time is saved which further minimizes the challenges in formulation development. In the current chapter, physico-chemical properties of the drugs received will be tested along with their compatibility with other components of the formulation.(1)

4.2. Materials and Methods

4.2.1. Materials

TAC and FBX were recieved as gift samples from Concord Biotech Ltd., India and Ami Drugs and Specialty Chemicals, India, respectively. Glyceryl monooleate (GMO) was received as gift sample from Mohini Organics, India. Polyvinyl Alcohol (PVA)-6000 and lactose were purchased from Acros Organics, USA and Hi-Media Pvt. Ltd. India, respectively.

4.2.2. Authentication of Tacrolimus

TAC was characterized with help of melting point, UV absorption spectrum, FTIR (Fourier transform infrared) spectrum and differential scanning calorimetric (DSC) thermograms.

4.2.2.1. Melting point determination

For the determination of melting point of TAC, a thin-walled capillary tube was taken and was sealed at one end using a Bunsen flame. Then, TAC was taken in an amount sufficient to form a dense packing of 3 mm height, and was filled in the tube by gently tapping its bottom. This packed capillary was attached to normal mercury thermometer and immersed in a Thiele tube filled with oil bath. This oil was heated in a controlled manner by moving the Bunsen burner back and forth along the side arm of Thiele tube which is designed to form convection currents allowing rapid heat

transfer and maintaining uniform temperature throughout the oil.(2) The temperature range within which TAC became a liquid was recorded and was reported as its melting point range.

4.2.2.2. UV absorption spectrum

10 µg/ mL of standard solution of TAC was prepared in acetonitrile. This solution was scanned over 200-400 nm wavelength (λ) with UV Visible spectrophotometer against methanol as blank.(3, 4)

4.2.2.3. Fourier transform infrared (FT-IR) spectrum

For performing FT-IR, sample preparation was done by Potassium bromide (KBr) pellet method wherein, moisture-less KBr (previously dried in oven) was mixed with approximately 1-2 % of TAC sample taken in a mortar and ground into a fine powder using a pestle. This powder-mix was placed in stainless steel dye and compressed in to a pellet using a hydraulic press. The resulting pellet was placed in Alpha FTIR spectrophotometer (Bruker, USA), scanned over the range of 4000-500 cm^{-1} and recorded spectrum was observed for the presence of characteristic peaks.(5)

4.2.2.4. Differential Scanning Calorimetry (DSC)

For performing DSC, approximately 5 mg of TAC sample was mounted onto the pan ensuring its uniform distribution along the bottom of the pan. The sample pan was closed with the cover and closed with ample pressure avoiding its distortion. An empty sample pan was also sealed in similar way and utilized as reference pan. Test sample as well as reference pans were placed in respective sample holders of DSC-60 differential scanning calorimeter (Shimadzu, Japan) and the instrument was programmed to operate in a manner where the instrument would heat 10°C every min working in range of 30-300°C under inert environment with the help of nitrogen atmosphere. DSC thermogram was observed for characteristic endothermic peak.(6)

4.2.2.5. Solubility study

Solubility of TAC was found out in various solvents and lipids 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 isolate undissolved drug and a supernatant was collected and analyzed using HPLC analytical method as described in section 3.4.1 after suitable dilution as required.

4.2.3. Authentication of Febuxostat

FBX was characterised for its melting point, UV absorption spectrum, FT-IR spectrum and DSC thermograms as described under section 4.2.3.1, 4.2.3.2, 4.2.3.3, and 4.2.3.4.

4.2.3.1. Melting point determination

For the determination of melting point, a thin-walled capillary tube was sealed at one end using a Bunsen flame and the FBX sample, sufficient to form dense packing of 3 mm height, was filled in the tube by gently tapping its bottom. This packed capillary was attached to normal mercury thermometer and immersed in a Thiele tube filled with oil bath. The oil was heated in a controlled way by moving the Bunsen burner back and forth along the side arm of Thiele tube which is designed to form convection currents allowing rapid heat transfer and maintaining uniform temperature throughout the oil.(2) The temperature range within which FBX became a liquid was recorded as its melting point range.

4.2.3.2. UV absorption spectrum

10 µg/ mL of standard solution of FBX was prepared in methanol using similar procedure as mentioned in chapter 3 section 3.5.2. This solution was scanned over 200-400 nm wavelength (λ) using UV Visible spectrophotometer against methanol as blank. An absorption spectrum was observed for presence of characteristic peaks at 315 nm wavelength.(7)

4.2.3.3. Fourier transform infrared (FT-IR) spectrum

For performing FT-IR, sample preparation was done by Potassium bromide (KBr) pellet method wherein moisture-less KBr (previously dried in oven) was mixed with approximately 1-2 % FBX sample in a mortar and ground into a fine powder using pestle. This powder-mix was placed in stainless steel dye and compressed in to a pellet using a hydraulic press. The resulting pellet was placed in Alpha FTIR

spectrophotometer (Brukar, USA), scanned over the range of 4000-500 cm^{-1} and recorded spectrum was observed for presence of characteristic peaks.(8)

4.2.3.4. Differential scanning calorimetry (DSC)

For performing DSC, approximately 5 mg of FBX sample was placed into the sample pan ensuring its uniform distribution along the bottom of the pan. The pan was closed with the cover and closed with ample pressure avoiding its distortion. An empty sample pan was also sealed in similar way and utilized as reference pan. These sample as well as reference pans were placed in respective sample holders of DSC-60 differential scanning calorimeter (Shimadzu, Japan) and the instrument was programmed to operate in a manner where the instrument would heat 10°C every min working in range of 30-300°C under inert environment with the help of nitrogen atmosphere. DSC thermogram was observed for characteristic endothermic peak.(9)

4.2.3.5. Solubility

Solubility of FBX was found out in various solvents and lipids 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 isolate undissolved FBX and supernatant was collected and analyzed using UV spectrophotometer as described in section 3.5.2 after suitable dilution as required.

4.2.4. Drug-excipient compatibility(10)

Multi-component 'prototype' formulation method was utilized for the analysis of compatibility among the drug and other formulation components. For the analysis, a blend of the respective drug and other excipients, beyond their maximum anticipated levels in the formulations (Table 4.1A), was prepared and their FTIR spectra were compared with that of individual formulation components. Table 4.1B represent the mixture component used for the DSC analysis. Glyceryl Monooleate was not used in mixture component analyzed by DSC due to its liquid nature.

Table 4.1A: Mixture components and their ratio for compatibility evaluation

Mixture component	Weight (mg)	
	TAC	FBX
TAC	10	-
FBX	-	10
Glyceryl monooleate	200	200
PVA	200	200
Lactose	200	200

Table 4.1B: Mixture components and their ratio for compatibility evaluation

Mixture component	Weight (mg)	
	TAC	FBX
TAC	10	-
FBX	-	10
PVA	200	200
Lactose	200	200

4.3. Results and Discussion

4.3.1. Tacrolimus Characterization

4.3.1.1. Melting point determination

A solid melts when the thermal energy overcomes the intermolecular forces that hold the solid together and the magnitude of these intermolecular forces depend on the chemical structure of the molecules. The melting point of TAC was reported to be 125 °C with a melting point range of 124 °C - 128 °C. The melting point was found to be 126 °C. This value was in accordance with value mentioned in literature.(11)

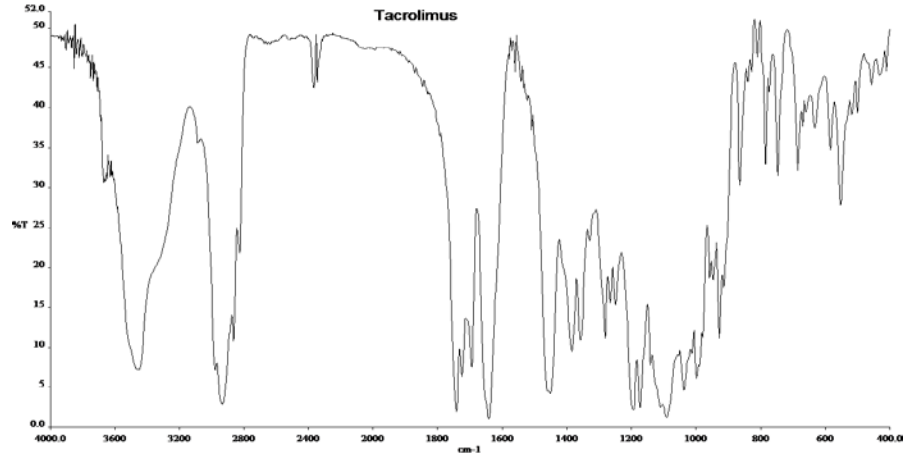
4.3.1.2. UV absorbance spectrum

TAC is freely soluble in most of the organic solvents like methanol, chloroform, ACN, acetone among others but it is experimentally insoluble in water. TAC is official in USP and according to USP, λ_{max} of TAC is 215 nm in UV(11). However,

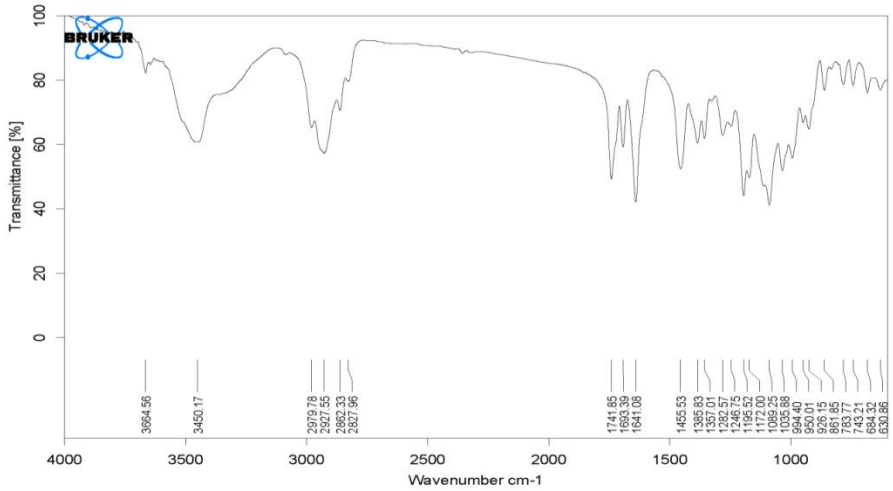
due to TACs insolubility in water and solubility in organic solvents like methanol and ACN which have solvent cut off wavelength at 205 nm and 195 nm respectively, interference occurs during the spectrum run of TAC by UV-Vis spectrophotometer(12). Thus, authentication of TAC was performed FT-IR and DSC.

4.3.1.3. Fourier transform infrared (FTIR) spectrum

A FTIR spectrum of TAC is presented in Fig. 4.1B. Characteristic peaks of TAC as reported in literature (Table 4.2), were observed in the FT-IR spectrum of drug sample received. Thus, result suggested the authenticity of the sample as TAC.



(A)



(B)

Figure 4.1: FT-IR spectrum (A) Reported FTIR spectra of TAC(13) (B) Obtained FTIR spectra of TAC

Table 4.2: Characteristic FT-IR spectrum bands of TAC

Structural characteristic	Spectrum bands	
	As per Literature(14)	Observed value
-OH	3650-3600	3450.17
-C-H-	3000-2850: stretch for aliphatic	2979.78, 2927.55, 2928.82, 2928.27
-C=O	1725-1705: Ketone 1750-1730: Ester	1741.85, 1693, 1641
-C-O-	1300-100: ether linkage	1089
C-N	1350-1000	1282.57

4.3.1.4. Differential scanning calorimetry (DSC)

DSC thermo gram of TAC is presented in fig. 4.2. A sharp endothermic peak was observed at 127.66 °C which suggests a crystalline anhydrous structure of TAC(11). Moreover, the reported melting point of the TAC is 126 °C which is close to a temp. of the endothermic peak. Thus, results confirmed the authenticity of the drug sample.

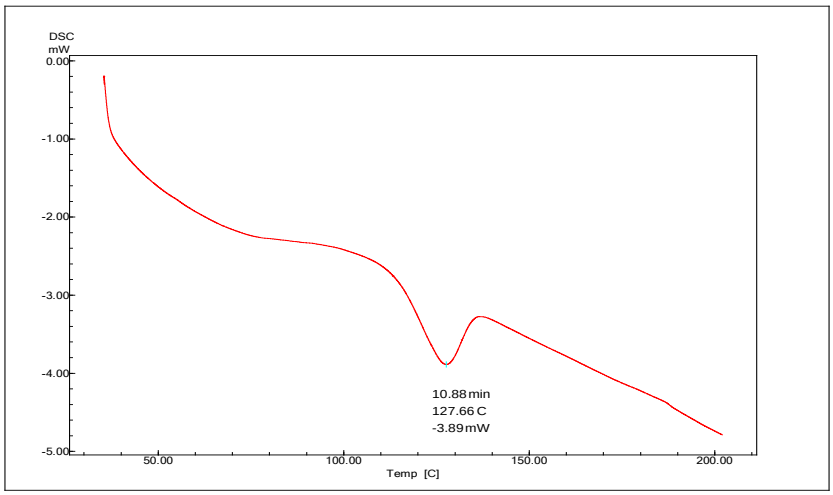


Figure 4.2: DSC thermogram of TAC

4.3.1.5. Solubility testing

TAC have maximum solubility in Acetonitrile i.e. 625.4 mg/mL respectively. Moreover, DMSO, ethanol, methanol and DMF also have a good solubility of TAC i.e. 94.16, 83.46, 75.82, and 29.78 respectively. Solubility study of TAC was also performed in GMO and GMS which was found approximately 40.32 and 32.81 mg/ml respectively as shown in fig. 4.3.

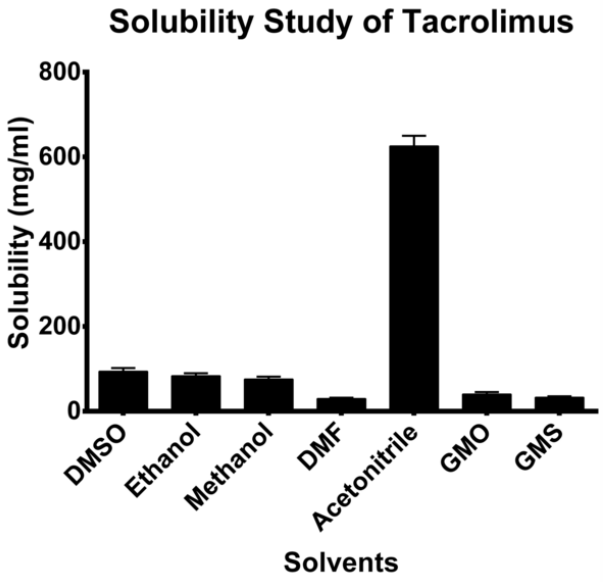


Figure 4.3: Solubility study of TAC in various solvent

4.3.2. Febuxostat Characterization

4.3.2.1. Melting point determination

A solid melts when the thermal energy overcomes the intermolecular forces that hold the solid together and the magnitude of these intermolecular forces depend on the chemical structure of the molecules. The melting point of FBX was reported to be 237 °C with a melting point range from 235 °C - 240 °C. The melting point was found to be 237 °C. This value is in accordance with value mentioned in literature.(15, 16)

4.3.2.2. UV absorbance spectrum

As shown in Fig. 4.4, the UV absorption spectrum of FBX sample in methanol showed peaks at 315 nm wavelength. The absorption maxima in above spectrum were found in accordance with that reported in literature(17). Presence of characteristic peaks suggested the authenticity of the sample as FBX.

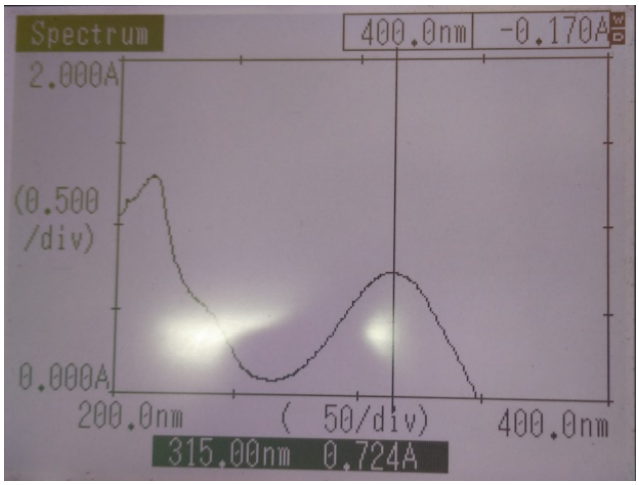
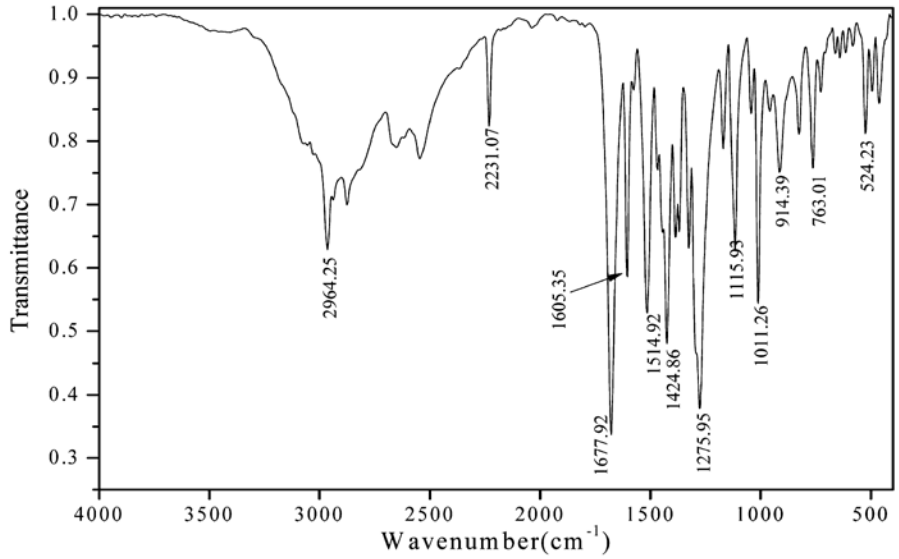


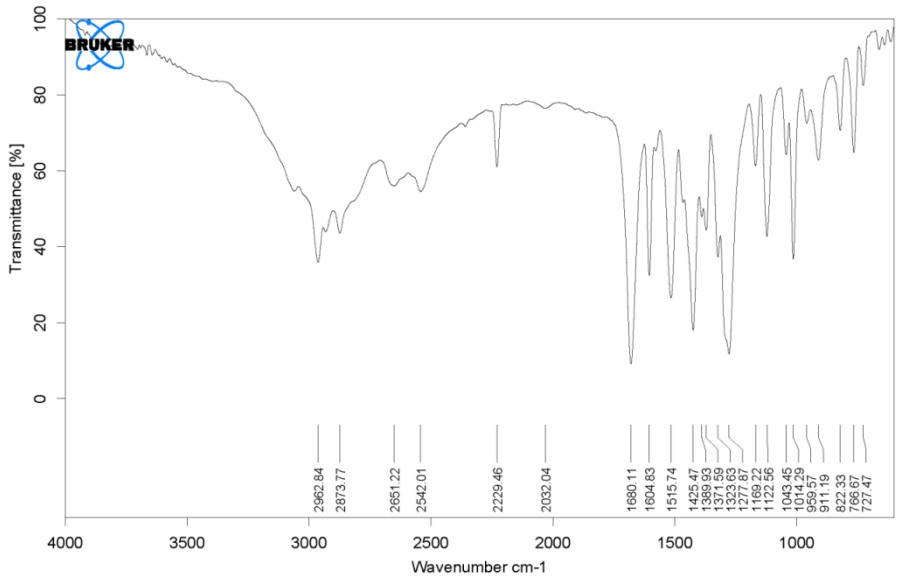
Figure 4.4: UV spectrum of FBX in methanol

4.3.2.3. Fourier transform infrared (FTIR) spectrum

A FTIR spectrum of FBX is presented in Fig. 4.5B. The characteristic peaks of FBX as reported in literatures (Table 4.3) were observed in the FT-IR spectrum of drug sample received. Thus, result suggested the authenticity of the sample of FBX.



(A)



(B)

Figure 4.5: FT-IR spectrum of FBX (A) Reported FTIR spectra of FTIR(18) (B) Obtained spectra of FTIR

Table 4.3: Characteristic FT-IR spectrum bands of FBX

Structural characteristic	Spectrum bands	
	As per Literature(14)	Observed value
-C-H	3150-3050: aromatic stretching 3000-2850: aliphatic stretching	2962.84, 2873.77
-OH	3400-2400	2654.22
-C=O	1750-1700	1680.11
-C-O	1300-1000	1277.87
-C≡N	2260-2240	2229.46

4.3.2.4. Differential scanning calorimetry (DSC)

A DSC thermogram of FBX is presented in fig 4.6. Sharp endothermic peak was observed at 207.42 °C which suggests crystalline anhydrous structure of FBX(16). Moreover, the reported melting point of the FBX is 209-212 °C which is close to the

temperature of the endothermic peak. Thus, results confirmed the authenticity of the drug sample.

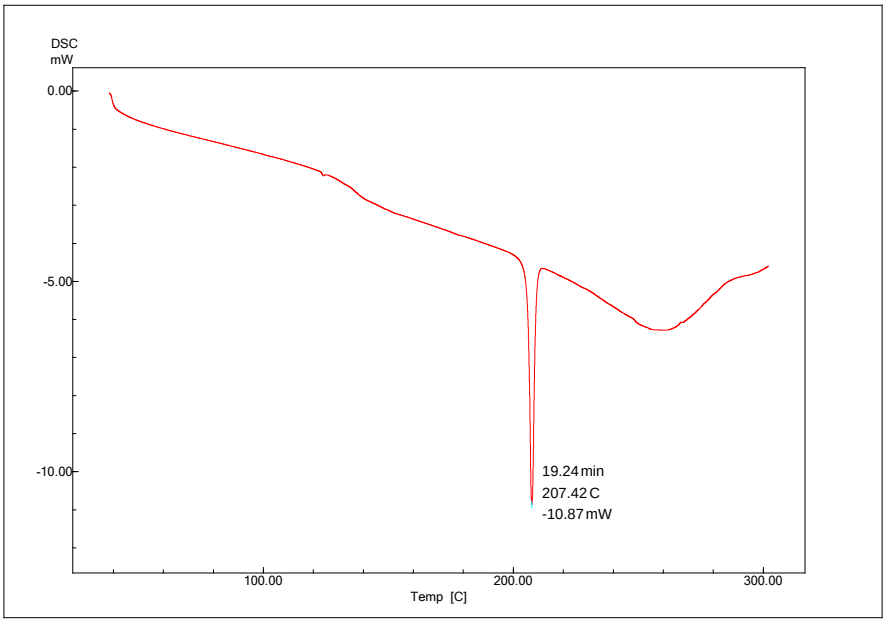


Figure 4.6: DSC thermogram of FBX

4.3.2.5. Solubility testing

FBX have maximum solubility in DMF i.e. 56.96 mg/mL, while least solubility in acetonitrile i.e. 7.34 mg/ml. Moreover, DMSO, ethanol, and methanol also have a good solubility of FBX i.e. 56.96, 26.34 and 34.61 respectively. Solubility study of FBX was also performed in GMO and GMS which was found approximately 48.04 and 36.14 mg/ml respectively as shown in fig 4.7.

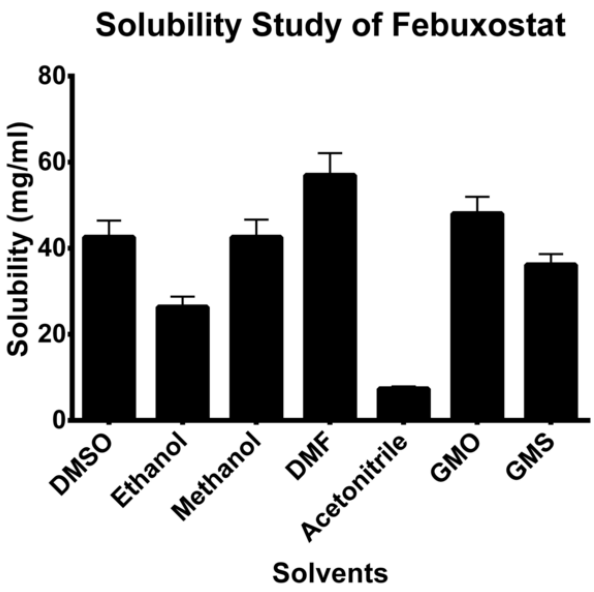
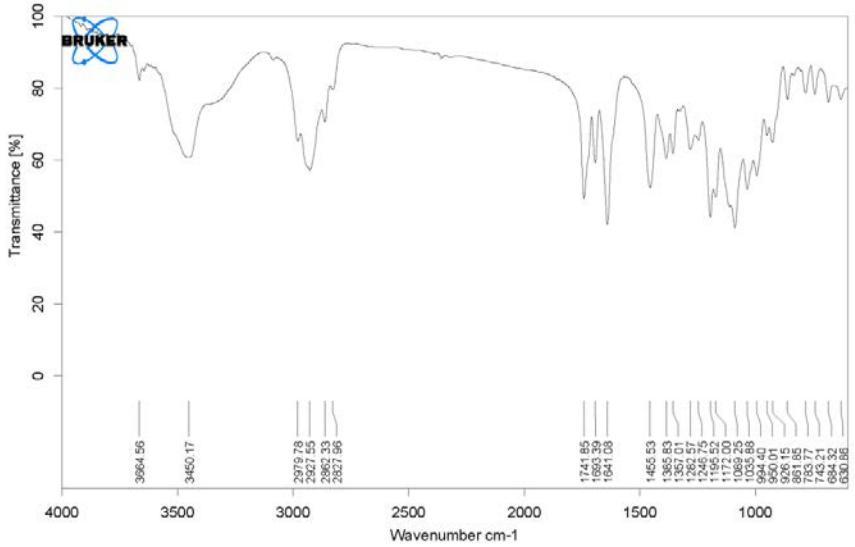


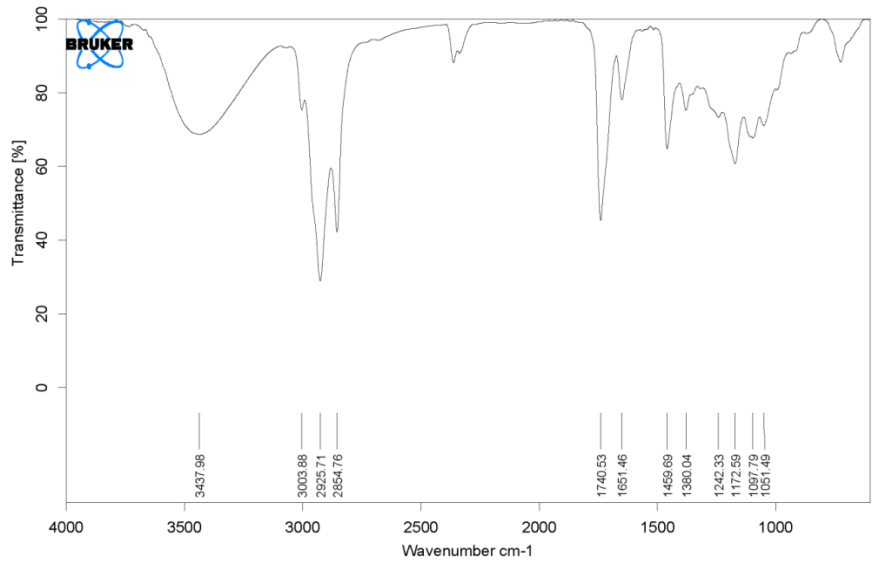
Figure 4.7: Solubility study of FBX in various solvent

4.3.3. Drug-excipient compatibility

Fig. 4.8 and 4.9 shows FTIR spectrum of drug (TAC/FBX) and physical mixture of drug (TAC/FBX) and excipients. Presence of all characteristic peaks in drug + physical mixture of excipients indicate the compatibility of drug with all excipients used in formulation preparation.

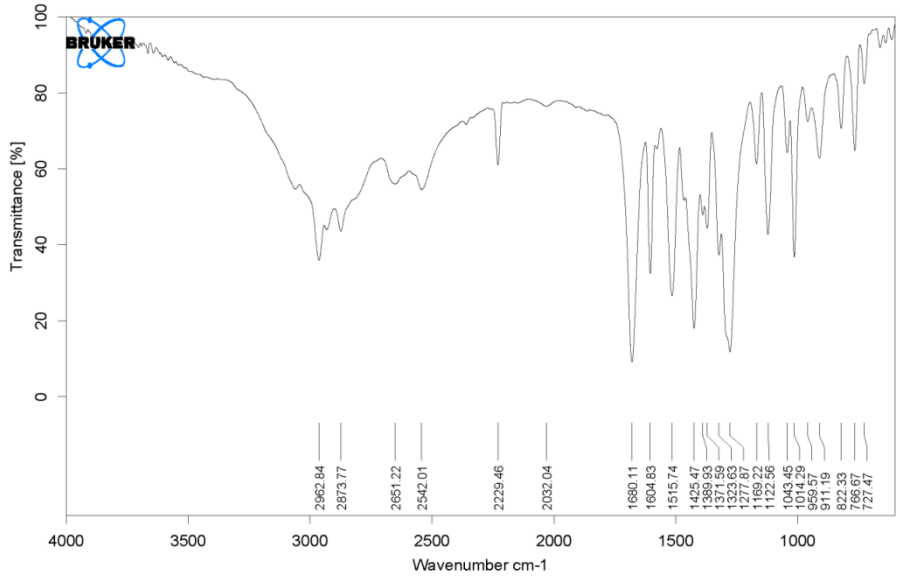


(A)

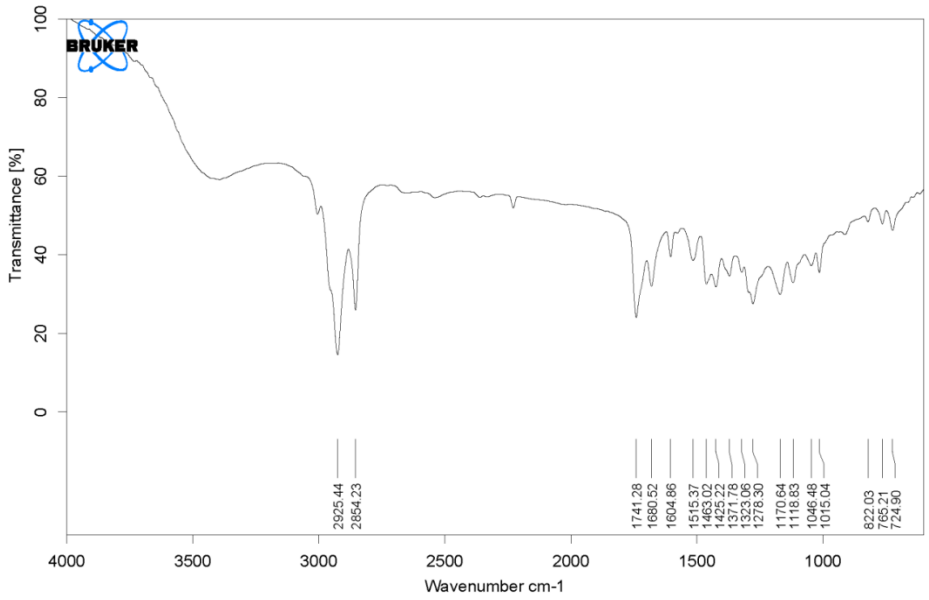


(B)

Figure 4.8: (A) FTIR Spectra of TAC (B) FTIR spectra of mixture of TAC, Glyceryl Monooleate, Polyvinyl Alcohol, Lactose



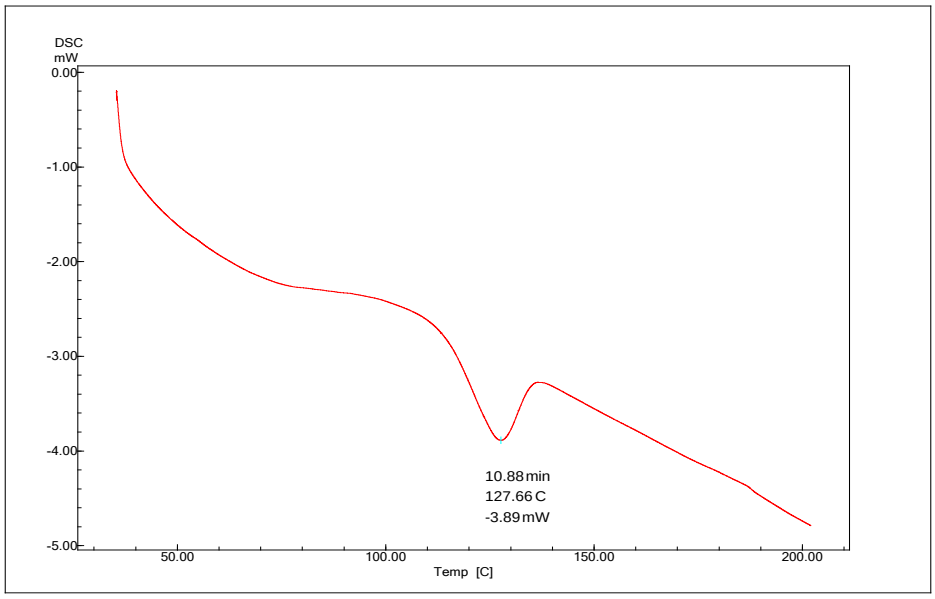
(A)



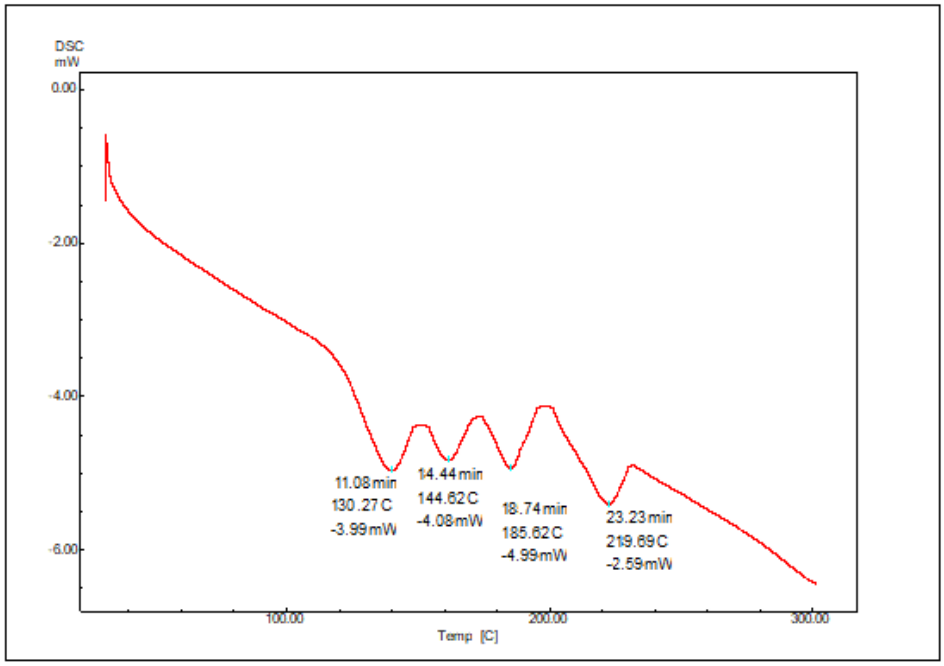
(B)

Figure 4.9: (A) FTIR spectra of FBX (B) FTIR spectra of FBX, Glyceryl Monooleate, Polyvinyl Alcohol, Lactose

Fig. 4.10 and 4.11 shows the DSC thermogram of drug (TAC/FBX) and physical mixture of drug (TAC/FBX) and excipients. Presence of all characteristic peaks in drug + physical mixture of excipients indicate the compatibility of drug with all excipients used in formulation preparation.

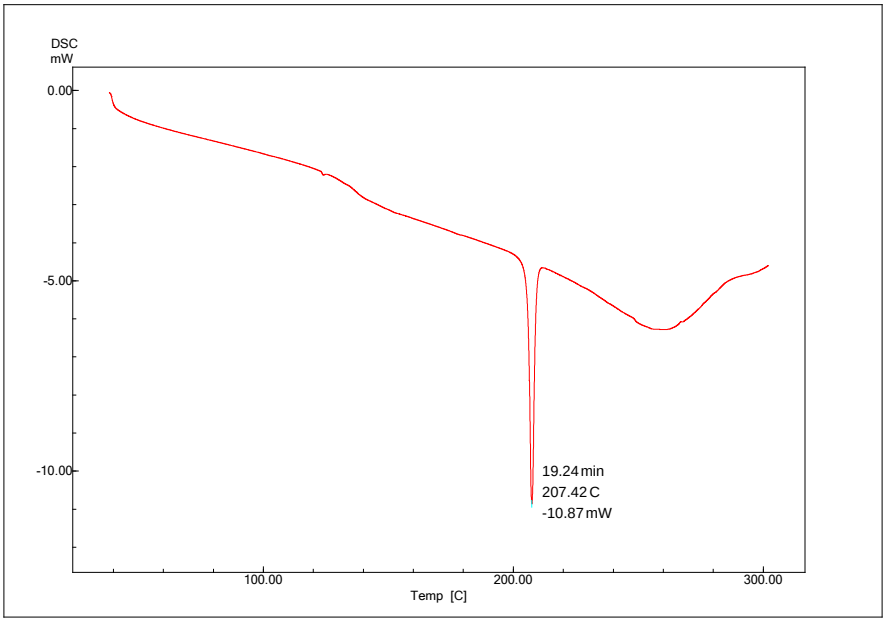


(A)

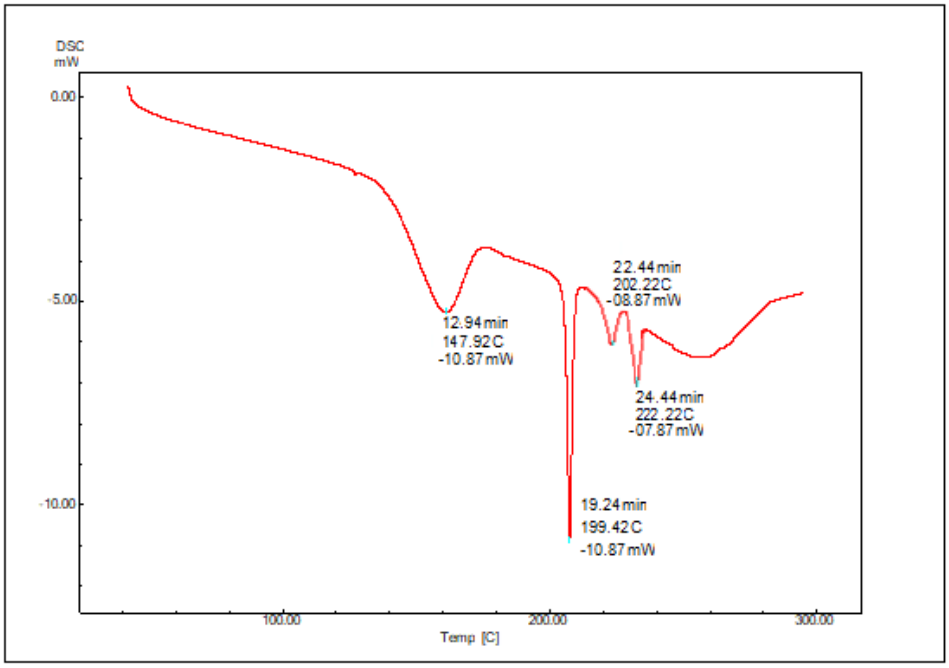


(B)

Figure 4.10: (A) DSC thermogram of TAC (B) DSC thermogram of mixture of TAC, Polyvinyl Alcohol, Lactose



(A)



(B)

Figure 4.11: (A) DSC thermogram of FBX (B) DSC thermogram of FBX, Polyvinyl Alcohol, Lactose

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