Contents

4.1. INTRODUCTION

As the name suggests, preformulation refers to a group of studies conducted prior to formulation development. These studies are essential in providing the scientifically driven foundation for successful development of a robust dosage form with pre-defined characteristics. Such studies also help in saving time and money by minimizing the challenges during formulation development.

Present chapter includes the authentication of drugs received and their compatibility testing with other formulation components of interest.

4.2. MATERIALS & METHODS

4.2.1. Materials

Vinpocetine was obtained as a gift sample from Covex S.A., Madrid, Spain. Noopept was purchased from Nootrico SA, New York, United States. PLGA (lactide/glycolide ratio 50:50) was a generous gift from Purac Biomaterials, Netherlands. Phospholipon 90H was kindly gifted by Lipoid GmBH, Germany. Poloxamer® 188 was purchased from BASF, India. Sodium deoxycholate was purchased from S.D. Fine Chemicals, Mumbai, India.

4.2.2. Authentication of Vinpocetine

Vinpocetine was authenticated for its melting point, UV absorption spectrum, fourier transform infrared (FT-IR) spectrum and differential scanning calorimetric (DSC) thermograms.

4.2.2.1. Melting point determination

A thin-walled capillary tube was sealed at one end using a Bunsen flame and the Vinpocetine 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. The temperature range within which VPN became a liquid was recorded as its melting point range.

4.2.2.2. UV absorption spectrum

Standard solution of VPN (10 μg/mL) was prepared in 96% ethanol using similar method as described in chapter 3 section 3.5.1. This solution was scanned over a wavelength (λ) range of 200-400 nm using UV Visible spectrophotometer against 96% ethanol as blank. The absorption spectrum was observed for presence of characteristic peaks at 229, 275 and 315 nm wavelengths [1].

4.2.2.3. Fourier transform Infrared (FT-IR) spectrum

Potassium bromide (KBr) pellet method was used for sample preparation where moisture-less KBr (previously dried in oven) was mixed with approximately 1-2 % VPN 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 (Bruker Optics, USA), scanned over the range of 4000-500 cm^{-1} and recorded spectrum was observed for presence of characteristic peaks.

4.2.2.4. Differential scanning calorimetry (DSC)

Approximately 5 mg of VPN sample was placed into the sample pan ensuring its uniform distribution along the bottom of the pan. The sample pan was covered with the lid and sealed 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 at a heating rate of 10°C per minute in the range of 30-300°C under inert nitrogen atmosphere. DSC thermogram was observed for characteristic endothermic peak.

4.2.3. Authentication of Noopept

Noopept was also authenticated for its melting point, UV absorption spectrum, fourier transform infrared (FT-IR) spectrum and differential scanning calorimetric (DSC) thermogram.

4.2.3.1. Melting point determination

A thin-walled capillary tube was sealed at one end using a Bunsen flame and NPT 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. The temperature range within which NPT became a liquid was recorded as its melting point range.

4.2.3.2. UV absorption spectrum

Standard solution of NPT (1000 μg/mL) was prepared in 95% ethanol. Briefly, accurately weighed 10 mg of NPT was transferred to a 10 ml calibrated volumetric flask and dissolved in 95% ethanol. The volume was made up to 10 ml with the same solvent to get 1000 μg/ml of standard NPT solutions. This standard solution was scanned over a wavelength (λ) range of 200-400 nm using UV Visible spectrophotometer against 95% ethanol as blank. The absorption spectrum was observed for presence of characteristic peaks at 253, 259, and 265 nm wavelengths [2].

4.2.3.3. Fourier transform Infrared (FT-IR) spectrum

Potassium bromide (KBr) pellet method was used for sample preparation where moisture-less KBr (previously dried in oven) was mixed with approximately 1-2 % NPT 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 FTIR spectrophotometer, scanned over the range of 4000-500 cm^{-1} and recorded spectrum was observed for presence of characteristic peaks.

4.2.3.4. Differential scanning calorimetry (DSC)

Approximately 5 mg of NPT sample was placed into the sample pan ensuring its uniform distribution along the bottom of the pan. The sample pan was covered with the lid and sealed 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 at a heating rate of 10°C per minute in the range of 30-300°C

under inert nitrogen atmosphere. DSC thermogram was observed for characteristic endothermic peak.

4.2.4. Drug-excipients compatibility testing

The compatibility among drugs and other formulation components were analyzed using a multi-component 'prototype' formulation method. Wherein, a blend of drug and other excipients, beyond their maximum anticipated levels in the formulations (Table 4-1), were prepared and their DSC thermograms were compared with that of individual formulation components.

Table 4-1. Mixture components and their ratio for compatibility evaluation

Mixture components	Weight Ratio			
	VPN UDL	VPN PNP	NPT UDL	NPT PNP
Vinpocetine	1	1	-	-
Noopept	-	-	1	1
Phospholipon 90H	20	-	20	-
Sodium deoxycholate	10	-	10	-
Poly lactic glycolic Acid	-	20	-	20
Poloxamer 188	-	10	-	10

4.3. RESULTS AND DISCUSSION

4.3.1. Vinpocetine authentication

4.3.1.1. Melting point determination

Melting occurs 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. Thus, compounds with different chemical structure tend to have different melting points which serve as their identification tool. The melting point of VPN was found to be 151 °C with a melting point range from 149 °C - 151 °C. The melting range was found in accordance with the value available in literature [1].

4.3.1.2. UV absorption spectrum

As shown in Fig. 4-1, the UV absorption spectrum of VPN sample in 96% ethanol showed peaks at 229 nm, 275 nm and 315 nm wavelengths. The absorption maxima in above spectrum were found in accordance with that available in literature [1]. Presence of characteristic peaks suggested the authenticity of the sample as VPN.

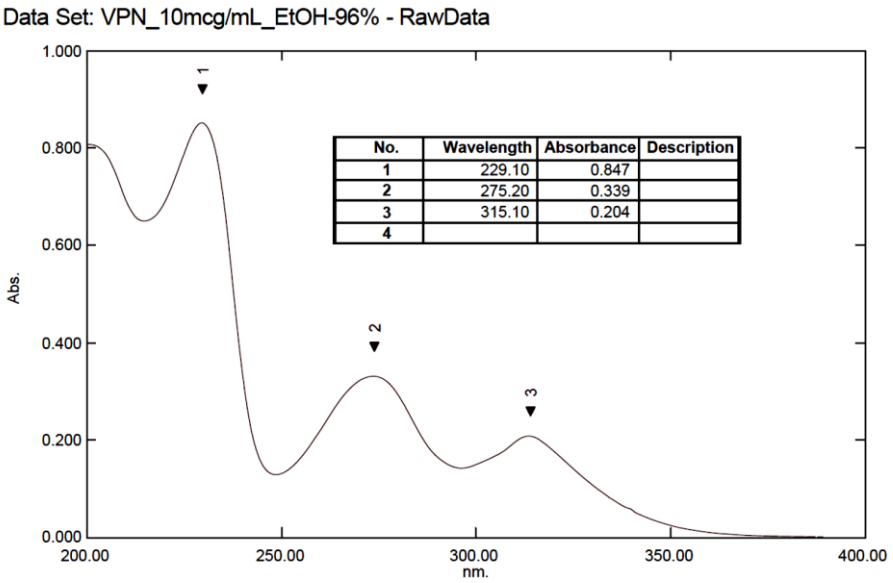

Fig. 4-1. UV absorption spectrum of Vinpocetine in 96% Ethanol

4.3.1.3. Fourier transform Infrared (FT-IR) spectrum

The FT-IR spectrum of VPN sample is presented in **Fig. 4-2**.

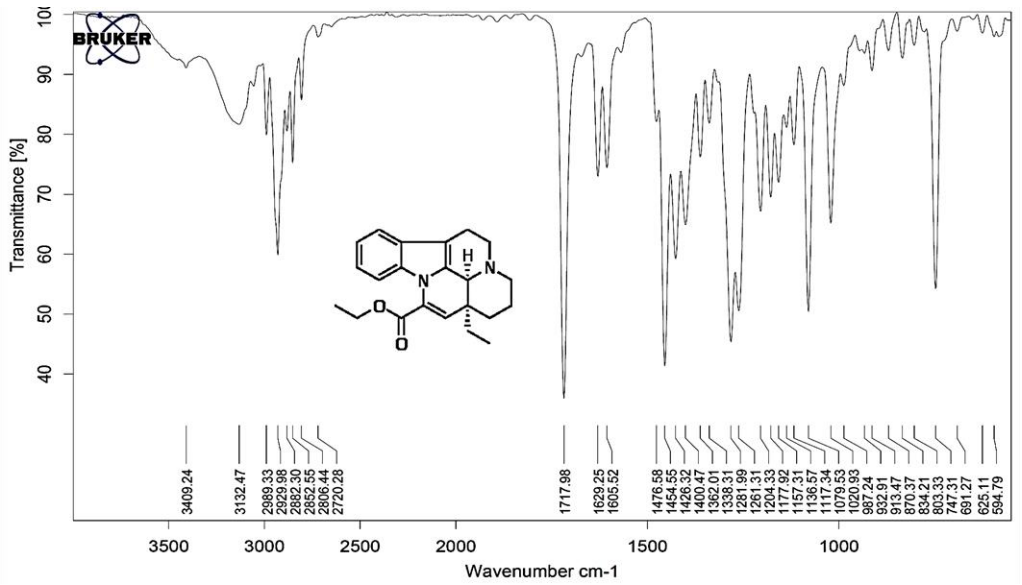

Fig. 4-2. FT-IR spectrum of Vinpocetine in KBr pellet

Table 4-2. Characteristic FT-IR absorption bands of Vinpocetine in KBr pellet

Structural characteristics	Absorption bands (cm^{-1})	
	Literature values [3, 4]	Observed values
aromatic stretching	2840-3000	2852-2989
carbonyl stretching (C=O)	1715	1718
ether stretching (C–O–C)	1020	1021

The characteristic peaks of VPN as reported in literatures (**Table 4-2**), were observed in the FT-IR spectrum of drug sample received. Thus, result suggested the authenticity of the sample as VPN.

4.3.1.4. Differential scanning calorimetry (DSC)

The DSC thermogram of VPN sample is presented in **Fig. 4-3**.

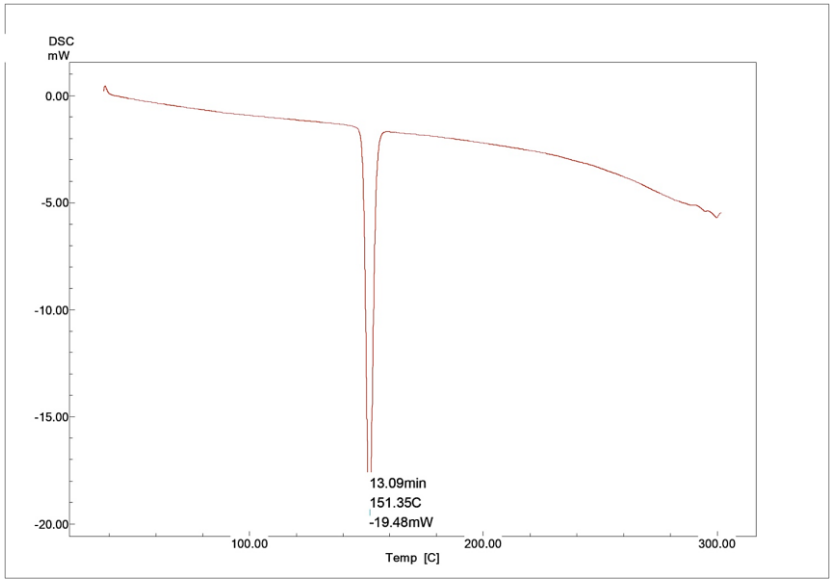

Fig. 4-3. DSC thermogram of Vinpocetine

A sharp endothermic peak was observed at 151.35 °C which corresponded to the crystalline anhydrous structure of VPN as available in literature [4]. Thus, results confirmed the authenticity of the sample as VPN.

4.3.2. Noopept authentication

4.3.2.1. Melting point determination

The melting point of NPT was found to be 97 °C with a melting point range from 95 °C - 97 °C. The observed melting point range was in line with the reported literature values [2].

4.3.2.2. UV absorption spectrum

As shown in **Fig. 4-4**, the UV absorption spectrum of NPT sample in 95% ethanol showed peaks at 253 nm, 259 nm and 265 nm wavelengths.

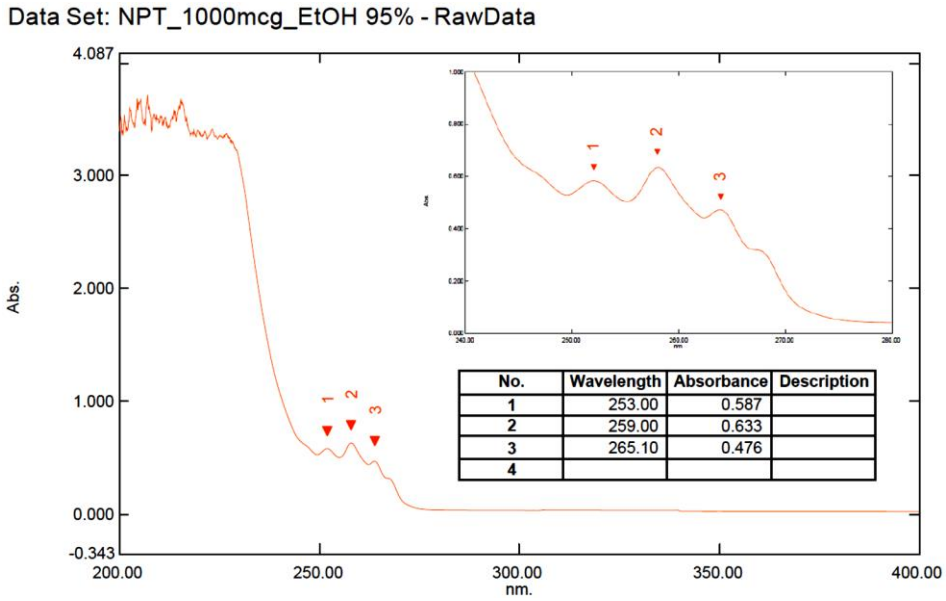

Fig. 4-4. UV absorption spectrum of Noopept in 95% Ethanol

The absorption maxima in above spectrum were found in accordance with that available in literature [2]. Presence of characteristic peaks suggested the authenticity of the sample as NPT.

4.3.2.3. Fourier transform Infrared (FT-IR) spectrum

The FT-IR spectrum of NPT sample is presented in **Fig. 4-5**.

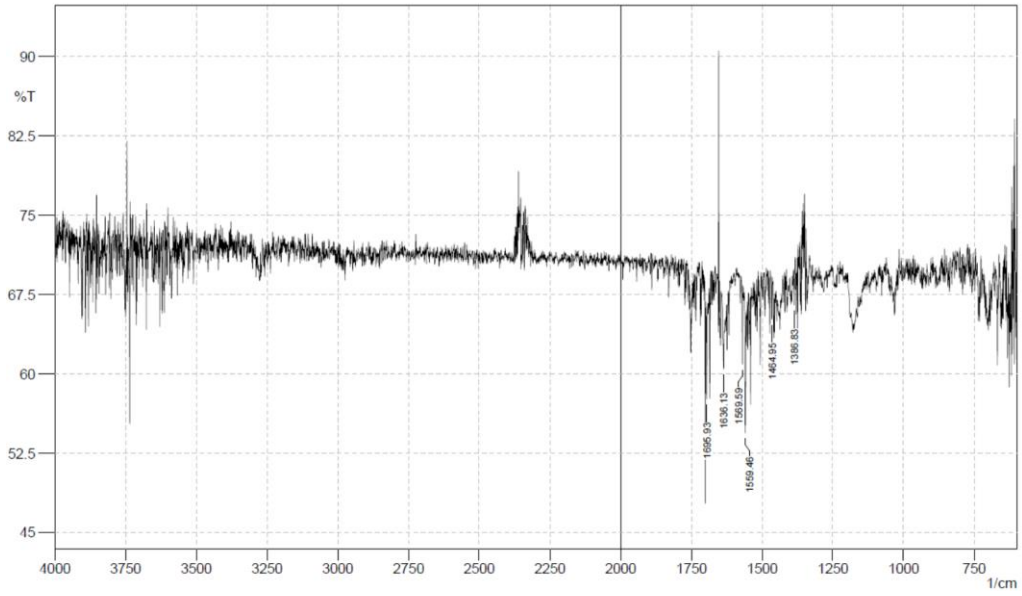

Fig. 4-5. FT-IR spectrum of Noopept in KBr pellet

Table 4-3. Characteristic FT-IR absorption bands of Noopept in KBr pellet

Structural characteristics	Absorption bands (cm–1)	
	Literature values [2]	Observed values
ester carbonyl (–$COOC_2H_5$)	1754	1752
peptide carbonyl (–CONH)	1695	1696
N-acyl carbonyl (N–CO–)	1636	1636

The characteristic peaks of NPT 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 as NPT.

4.3.2.4. Differential scanning calorimetry (DSC)

The DSC thermogram of NPT sample is presented in **Fig. 4-6**.

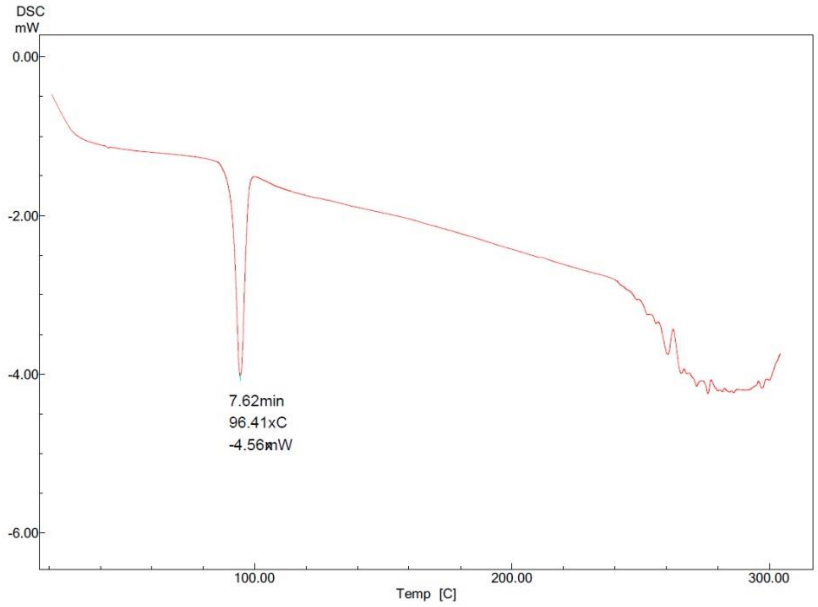

Fig. 4-6. DSC thermogram of Noopept

A sharp endothermic peak was observed at 96.4 °C which corresponded to the crystalline anhydrous structure of NPT as available in literature. Thus, results confirmed the authenticity of the sample as NPT.

4.3.3. Drug-excipients compatibility testing

Fig. 4-7 to **Fig. 4-10** show the DSC thermograms of drug-excipients mixture together with their individual components for VPN loaded UDL, VPN loaded PNP, NPT loaded UDL and NPT loaded PNP, respectively.

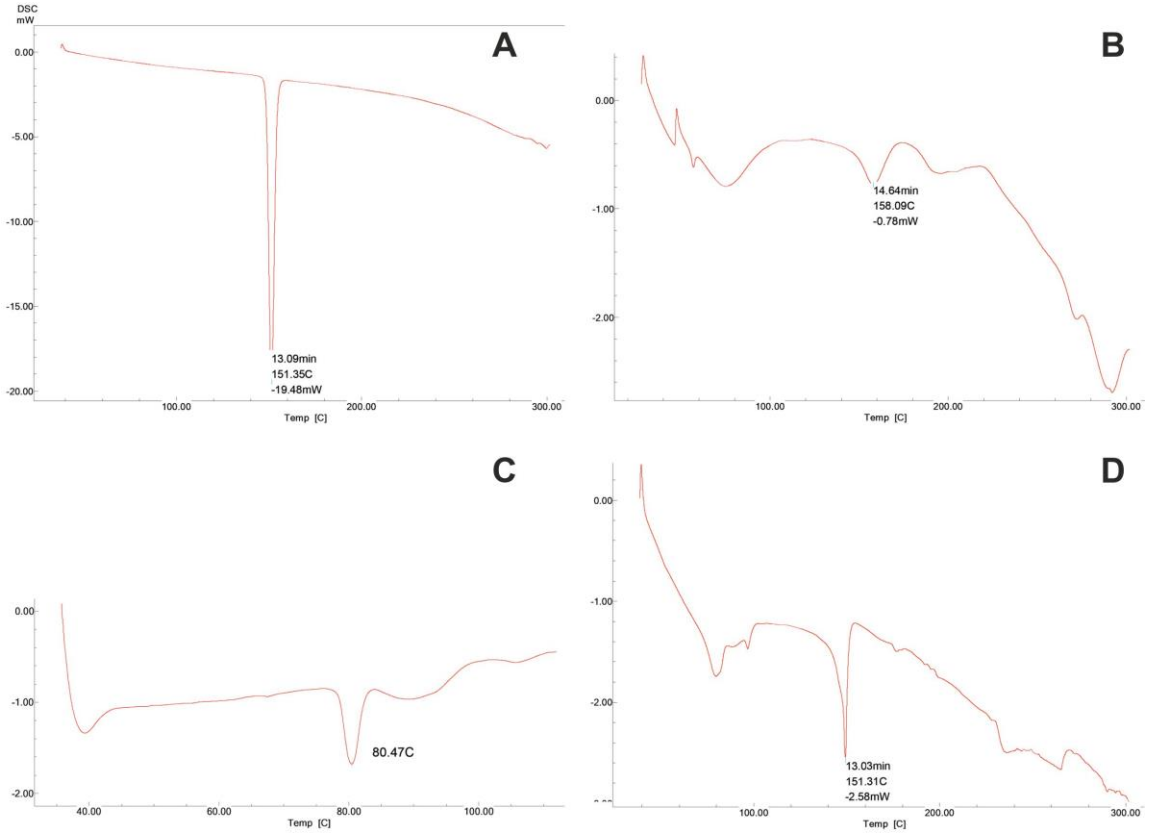

Fig. 4-7. DSC thermograms of A) Vinpocetine; B) Sodium deoxycholate; C) Phospholipon 90H and D) their mixture

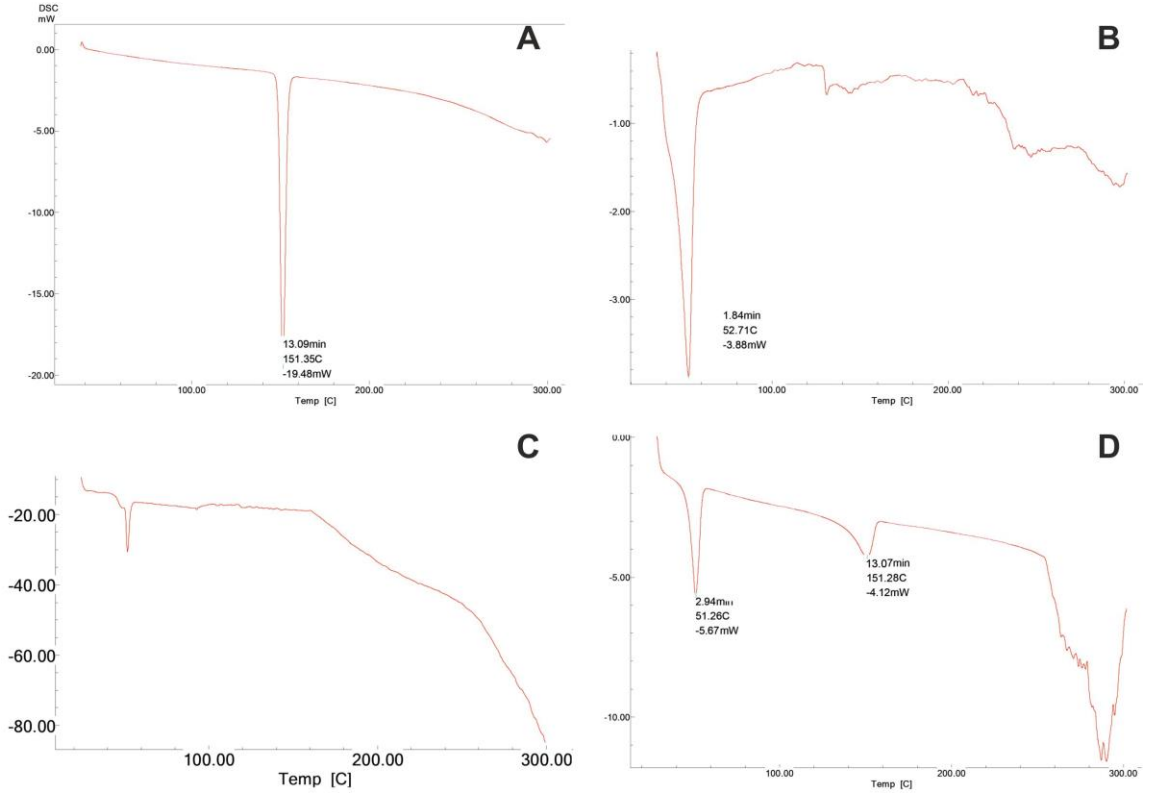

Fig. 4-8. DSC thermograms of A) Vinpocetine; B) Poloxamer 188; C) Poly lactic-glycolic acid and D) their mixture

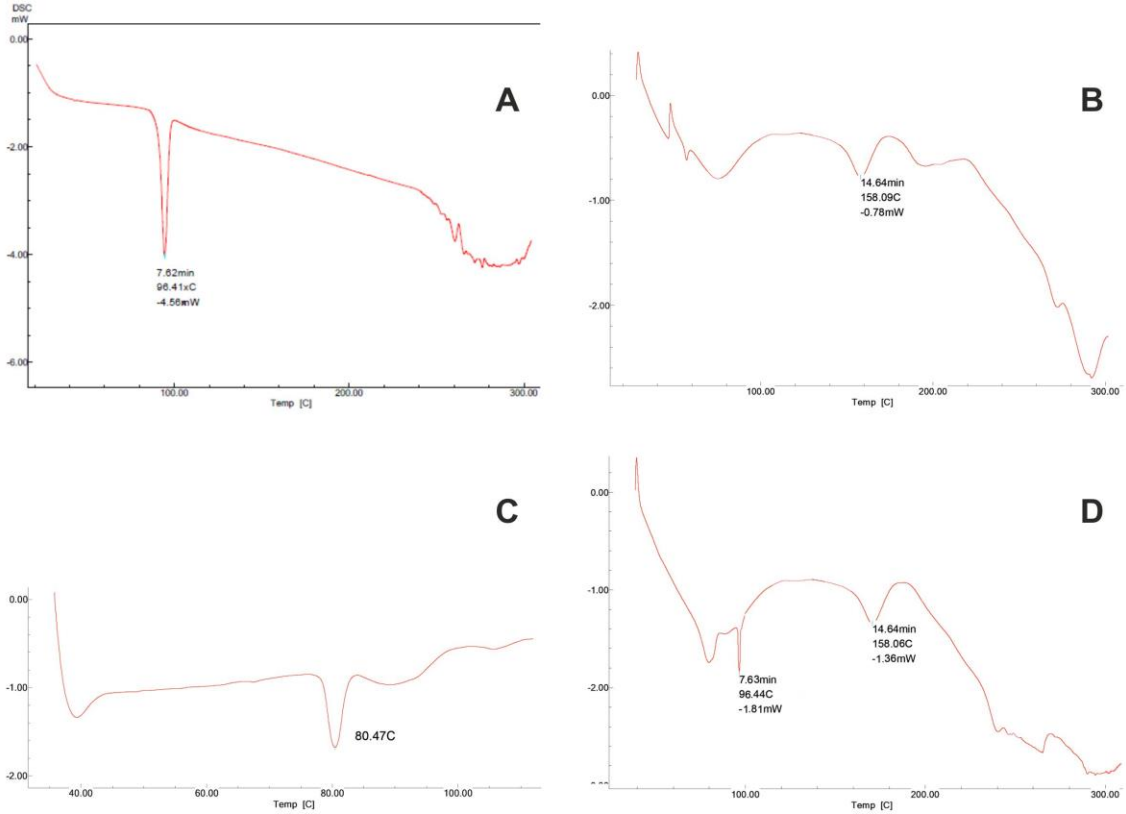

Fig. 4-9. DSC thermograms of A) Noopept; B) Sodium deoxycholate; C) Phospholipon 90H and D) their mixture

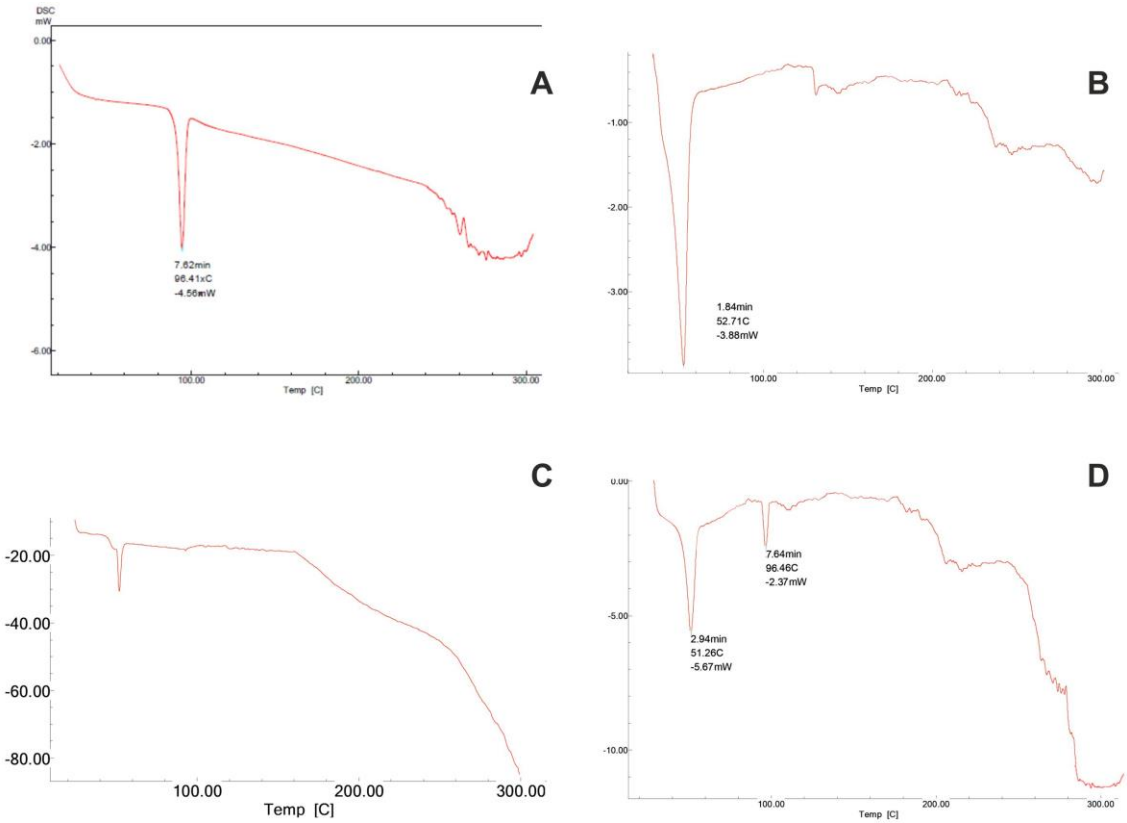

Fig. 4-10. DSC thermograms of A) Noopept; B) Poloxamer 188; C) Poly lactic-glycolic acid and D) their mixture

No shifts were observed in drugs' endothermic peaks in all four formulation prototype mixtures suggesting the compatibility among the mixture components.

References

1. *Vinpocetine Monograph*. Thirteenth ed. The Merck Index. New Jersey, USA: Merck & Co., Inc.
2. Gusev, A.V., et al., *Developing analytical methods for the creation of the state reference sample of noopept.* Pharmaceutical Chemistry Journal, 2007. **41**(12): p. 666-669.
3. Nie, S., et al., *In vitro and in vivo studies on the complexes of vinpocetine with hydroxypropyl-beta-cyclodextrin.* Arch Pharm Res, 2007. **30**(8): p. 991-1001.
4. Ahmed, T.A., *Formulation and clinical investigation of optimized vinpocetine lyoplant-tabs: new strategy in development of buccal solid dosage form.* Drug Des Devel Ther, 2019. **13**: p. 205-220.