Chemistry Research Journal, 2020, 5(4):6-20

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Thermo analytical study and purity determination of Anti-diabetic drugs Linagliptin and Empagliflozin in drug substances

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Abstract The thermal behavior of anti-diabetic drugs Linagliptin (LNG) and Empagliflozin (EMP) in their pure form was examined using several thermal strategies. The thermo gravimetry (TGA), derivative thermo gravimetry (DrTGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) were used to study the thermal decomposition and purity of both drugs. Thermodynamic parameters such as; activation energy (E^*), frequency factor (A), reaction order (n), enthalpy (ΔH^*), entropy (ΔS^*) and Gibbs free energy change of the decomposition (ΔG^*) were calculated using different kinetic models.

The data exhibit that the thermal degradation of the studied drugs followed first order kinetic behavior. The results of experimental purity items obtained from the thermal analysis technique for the cited drugs in their drug substances such as; purity, melting point, loss on drying, ash content and infra-red spectrum were in agreement with those obtained from the reported method, where no significant difference was observed. Thermal analysis justifies its application in quality control of pharmaceutical compounds due to its simplicity, sensitivity, speed and low operational costs.

Keywords Thermal analysis; Linagliptin; Empagliflozin; Kinetic studies; Purity determination; Quality control; infra-red spectrum.

Introduction

Linagliptin (LNG) is chemically known as 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl) methyl] purine-2, 6-dione [1]. It is a DPP-4 (dipeptidyl peptidase-4) inhibitor used for treatment of type-II diabetes. LNG acts by blocking the action of DPP-4 enzyme that destroys the hormone GLP-1 which helps to increase insulin secretions and inhibits the release of glucagon resulting in decreasing the glucose level in the circulation [2,3]. Empagliflozin (EMP) is chemically known as (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol [1]. It is a sodium-glucose co-

transporter-2 (SGLT-2) inhibitor. So that it can be used for treatment of type-II diabetes by blocking the reabsorption of glucose in the kidneys and promoting the excretion of excess glucose in urine [4,5].
Figure (1) displays the chemical structures of LNG and EMP[1].

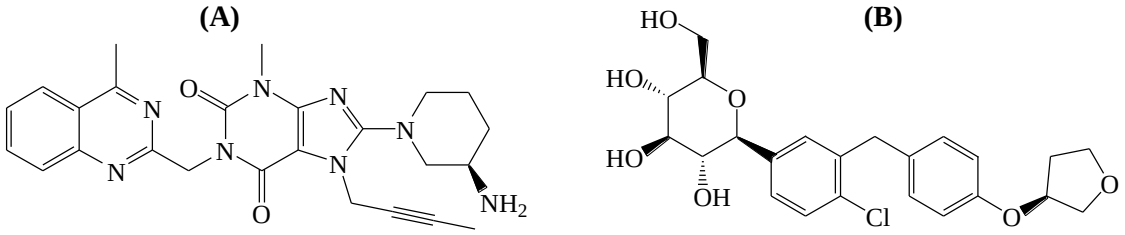

Figure 1: Chemical structures of LNG (A) and EMP (B)

Literature review basically covers the distinctive analytical strategies that have been developed for the determination of LNG including chromatographic [6-22], spectrophotometric [23–28] and spectrofluorimetric techniques [29]. On the other hand, chromatographic [30–41], spectrophotometric [42–45] and spectrofluorimetric techniques [46] have been accounted for quantitative analysis of EMP.

Thermal analysis is a group of techniques in which a physical property of a substance and/or its reaction products is subjected to a controlled temperature program that convey extremely sensitive measurement of heat change can be applied on a broad scale with pharmaceutical development. The increasing use of the combined techniques is providing more specific information, and thus this promotes more rapid explanation of the experimental curves obtained [47-50]. These methods find extensive use in quality control of drugs. These methods include:

1- ***Thermo-gravimetric Analysis (TGA)*** is a technique in which the amount of weight change of a substance is monitored either as a function of controlled temperature or isothermally as a function of time [51] as the temperature of the sample is increased in an atmosphere of N2, He, air or other gas, (TGA) is commonly employed in research and testing to determine degradation temperatures, absorbed moisture content of materials, decomposition and kinetic parameters, it is a fundamental laboratory instruments applied for investigation of the materials properties in various fields such as; pharmaceutical, environmental, food and petrochemical applications.[52-54]

2- ***Derivative Thermo-gravimetric Analysis (***DrTGA***)*** is a type of thermal analysis in which the rate of the materials weight changes upon heating is plotted against temperature and used to simplify reading the weight versus temperature thermo-gram peaks which occur close together.

3- ***Differential thermal analysis (DTA)*** is a common thermal analysis method in which an analyte and inert reference are heated at a certain heating rate while any temperature change is recorded the difference of temperature between the material under study and an inert reference (ΔT) is measured as a function of temperature (T), while the substance and reference are subjected to temperature program and changes in the sample, either exothermic or endothermic transitions as a function of temperature (T) can be detected relative to inert reference. DTA curve can be used as a finger print for identification purposes but usually the applications of this method are the determination of phase diagrams, heat change measurements and decomposition in various atmospheres [55-57], DTA is a popular tool used to characterize pharmaceutical substances, foods, organic and inorganic chemicals.

4- ***Differential scanning calorimetry (DSC)*** is a popular thermo-analytical technique which monitors the difference in the amount of required heat to increase the temperature of a sample and reference which should has an acceptable heat capacity in the range of scanned temperatures as a function of temperature. DSC is used in pharmaceutical industry as analytical tool of great importance for the identification and purity testing of active drugs, yielding results rapidly and efficiently [58-61]. It also applied for the quality control of raw materials used in pharmaceutical products [62-70].

In modern analytical laboratory, there is always a need for rapid and significant methods for identification and purity determination of drugs. The determination of the melting point using DSC method has been adequately used as a method of estimating the degree of purity of drugs [71].

In present work, thermal behavior of LNG and EMP were studied by using different techniques such as; TGA, DrTGA, DTA and DSC.

Experimental

Raw Materials

LNG (batch no. LIP0317004) manufactured by Lee pharma limited, Tenalanga, India; and is kindly supplied from Rameda pharmaceutical company, Egypt. Its purity was checked according to a reported method [14] and was found to be 99.40%. It was conducted using an Agilent-1260 series HPLC device using Agilent C18 column (150 mm × 4.6, 5µm) maintained at temperature 30^0C. A mobile phase consisting of phosphate buffer (pH3.4): acetonitrile (70:30 v/v) was used at a flow rate 1ml/min with UV detection at 240nm.

EMP (batch no. OT-EMP/001/115) manufactured by Optrix private limited, Tenalanga, India; and is kindly supplied from Hikma pharma company, Egypt. Its purity was checked according to a reported method [31] which was performed using an Agilent-1260 series HPLC device using Agilent C18 column (250 mm × 4.6, 5µm) maintained at ambient temperature. A mobile phase consisting of phosphate buffer (pH2.8): acetonitrile (45:55 v/v) was used at a flow rate 1ml/min with UV detection at 228nm, where the purity was found to be 99.20%.

Instrumentation and methods

Thermal analysis studies were made using simultaneous TGA-DTG thermal analyzer apparatus (Shimadzu DTG-60H) with TA 60 software in dry nitrogen atmosphere at a flow rate of 30 mL/min in platinum crucible with an empty platinum crucible as a reference. The experiments were performed between ambient and 800 °C. The temperature program had a heating rate 10 °C/min. α- Al_2O_3 was used as the reference material. Thermodynamic parameters such as; activation energy (E^*), frequency factor (A), reaction order (n), enthalpy (ΔH^*), entropy (ΔS^*) and Gibbs free energy change of the decomposition (ΔG^*) were obtained by using the Horowitz-Metzger and Coats-Redfern relations [72, 73].

DSC curves were measured on Shimadzu DSC-50 cell, in dynamic nitrogen atmosphere with a constant flow rate of 30 mL/min and heating rate of 2 °C/min; up to temperature 400 °C. The samples mass was approximately 2 mg of the drugs without any further treatment placed in a sealed aluminum pan. An empty aluminum pan was used as a reference. The purity determination was performed using heating rate of 10 °C/min in the temperature range from 25 to 400 °C in nitrogen atmosphere.

Melting points were measured using Stuart® melting point, Digital, Advanced, SMP30, USA (Serial no. R000101613).

Infra-red spectroscopy: IR spectra of both drugs were recorded using Nicolet 6700 FT-IR spectrometer from Thermo Scientific, USA, Which incorporates extensive capabilities for handling optical filters, polarizers and mirrors for specific applications through the OMNIC Software suite.

Results and discussion

Thermal analysis of LNG and EMP

Figure 2A shows the TGA, DrTGA and DTA curves of LNG. The TGA and DrTGA curves show that LNG is thermally stable up to 72.42°C and decomposes at higher temperatures in two steps. In the first step (72.42-450.74°C) LNG decomposes through the loss of $C_{13}H_{13}N_3$, (44.742%). LNG also continues to decompose through the second step by loss of $C_{12}H_{15}N_5O_2$, (55.233%).

The DTA curve shows an exothermic peak at 45.23°C which may be due to glass transition, an endothermic peak at 205.63°C which may be due to melting of LNG, and exothermic peaks appear during decomposition of the drug as follow: medium and broad peak at 266.93°C, a very weak and broad peak at 377.09°C, which may be attributed to the first decomposition step, and a very strong and broad peak at 590.04°C which may be attributed to the second decomposition step. The suggested thermal decomposition mechanism of LNG is shown in figure 2B.

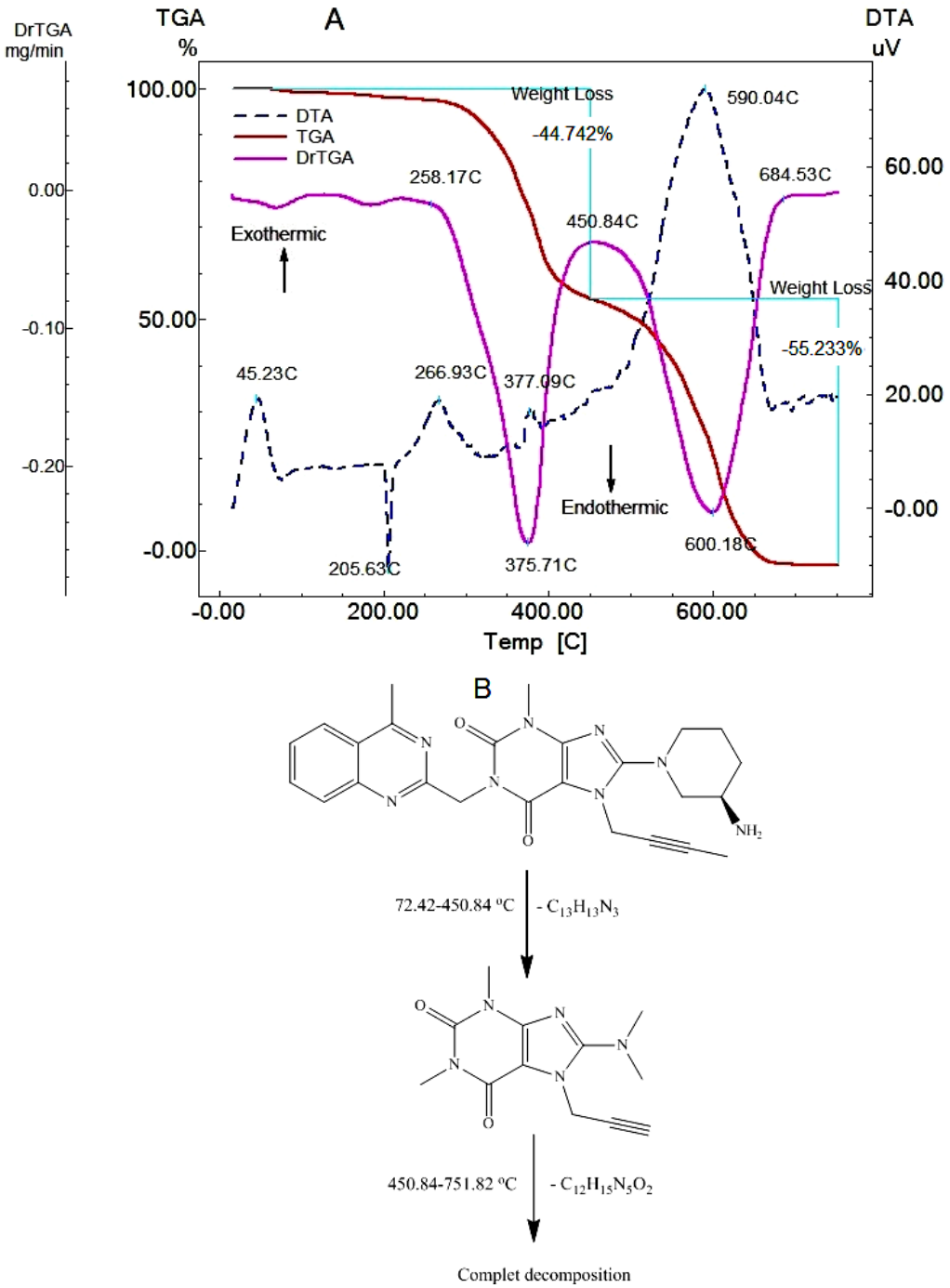

Figure 2: TGA, DrTGA and DTA curves of LNG (A) and suggested decomposition mechanism of LNG (B) at heating rate of 10°C/min.

Figure 3A shows the TGA, DrTGA and DTA curves of EMP. The TGA and DrTGA curves show that EMP is thermally stable up to 180.59°C and decomposes at higher temperatures in two steps. The first decomposition step occurs in the temperature range of 180.59°C-410.58°C, where EMP loses $C_{10}H_{12}O_2Cl$, (43.64%). The second decomposition step takes place in the temperature range of 410.58°C-700.00°C; where EMP loses $C_{13}H_{15}O_5$, (56.357%).

The DTA curve shows an exothermic peak at 49.97°C which may be due to glass transition, an endothermic peak at 154.47°C which may be due to melting of EMP, an extremely weak and broad exothermic peak at 363.89°C which may be attributed to the first decomposition step, and a very strong and broad exothermic peak at 532.81°C due to the second decomposition step. The suggested thermal decomposition mechanism of EMP is shown in figure 3B.

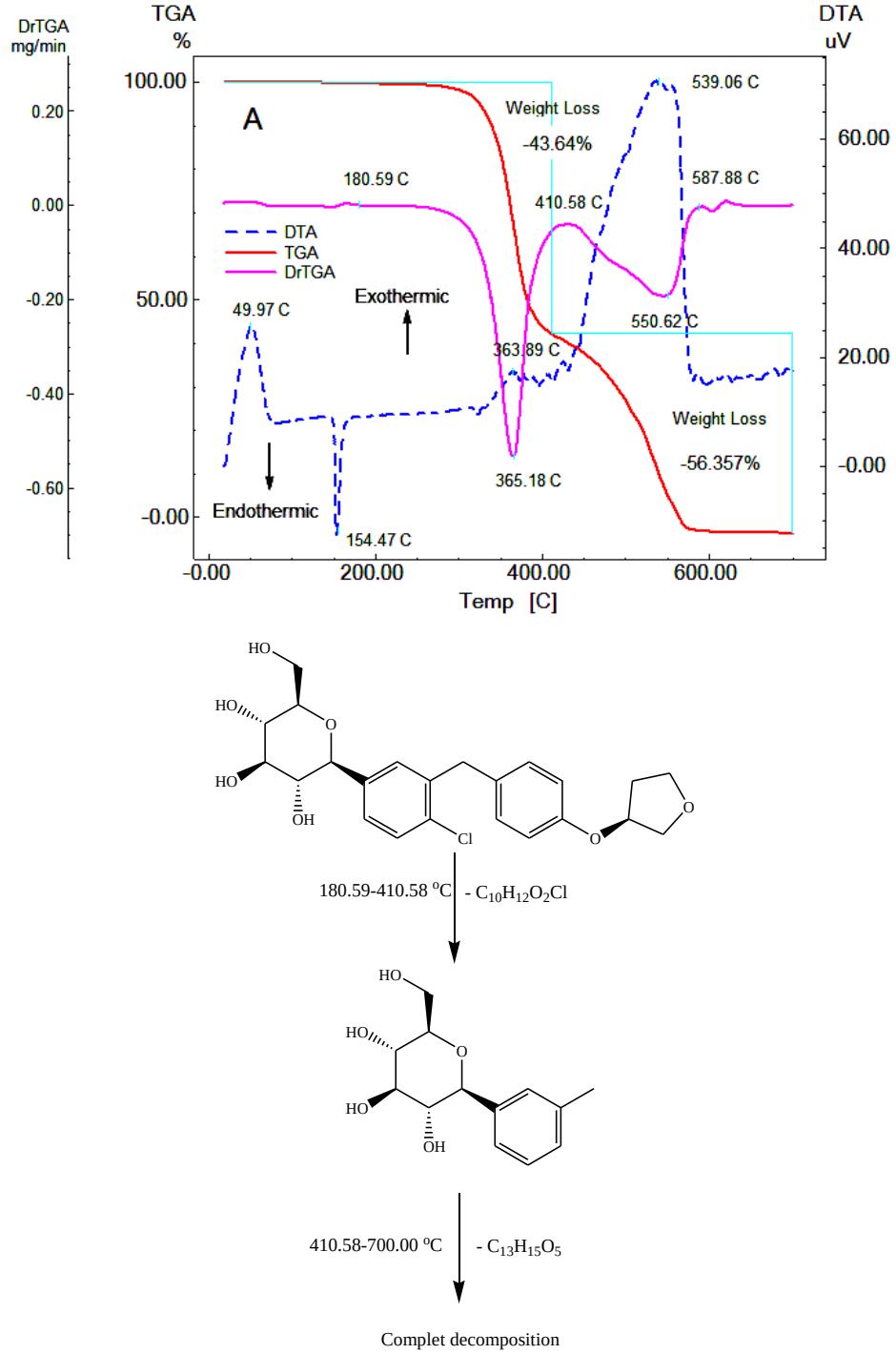

Figure 3: TGA, DrTGA and DTA curves of EMP (A) and suggested decomposition mechanism of EMP (B) at heating rate of 10°C/min.

Thermodynamic parameters of LNG and EMP

Thermodynamic parameters such as activation energy (E), enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG) were calculated using Horowitz-Metzger and Coats-Redfern methods [72, 73].

(i) Horowitz-Metzger method

Horowitz-Metzger equation can be represented as follow:

$$\log .[\log \frac{W_f}{W_f - W}] = \frac{\theta.E}{2.303RT_s^2} - \log 2.303 \quad (1)$$

Where W_f is the mass loss at the end of the decomposition process, W is the mass loss up to temperature T, R is the gas constant, T_s is the DrTGA peak temperature and $\theta = T-T_s$. A plot of log [log W_f / (W_f - W)] against θ would give a straight line and E could be calculated from the slope.

(ii) Coats-Redfern method

Coats-Redfern equation can be represented as follow:

$$\log\left(\frac{\log\left[W_f / W_f - W\right]}{T^2}\right) = \log\left[\frac{AR}{\phi E}\left(1-\frac{2RT}{E}\right)\right] - \frac{E}{2.303RT} \quad (2)$$

Where ϕ is the heating rate; since 1-2RT / E≅1, the plot of the left-hand side of equation (2) against 1/T would give a straight line, E was calculated from the slope and the Arrhenius constant (A) was obtained from the intercept. The entropy ΔS, enthalpy ΔH and free energy ΔG of activation were calculated using the following equations:

$\Delta S = 2.303 [\log (Ah / kT)] R$ (3)

$\Delta H = E^* - RT$ (4)

$\Delta G = H^* - T_s \Delta S^*$ (5)

Where k and h are the Boltzman and Planck constants; respectively

Table 1 shows thermodynamic parameters of thermal decomposition of LNG and EMP.

Table 1.Thermodynamic parameters of thermal decomposition of LNG and EMP

Temperature range (°C)	E (KJ mol^{-1}) HM (CR)	A (S^{-1}) HM (CR)	ΔS (J mol^{-1} K^{-1}) HM (CR)	ΔH (KJ mol^{-1}) HM (CR)	ΔG (KJ mol^{-1}) HM (CR)
LNG					
72.42-450.84	87.021 (65.553)	1.26 x 10^6 (5.66 x 10^5)	-134.583 (-141.272)	81.628 (60.156)	168.933 (151.800)
450.84-751.82	137.226 (65.70)	1.75 x 10^7 (4.21 x 10^3)	-115.2 (-184.4)	129.967 (57.805)	230.551 (218.897)
EMP					
180.59-410.58	177.017 (153.772)	8.06 x 10^{13} (5.77 x 10^{11})	14.988 (-26.085)	171.711 (148.462)	162.146 (165.109)
410.58-700.00	118.195 (108.382)	3.29 x 10^6 (1.84 x 10^6)	-128.630 (-133.435)	111.347 (101.529)	217.289 (211.429)

N.B. Kinetic parameters obtained by the methods of Horowitz-Metzger (HM) and Coats-Redfern (CR) for LNG and EMP.

Figures 4-7 show the Coats-Redfern and Horowitz-Metzger plots of the decomposition of LNG and EMP drugs.

Thermal analysis results exhibits that EMP is more thermally stable than LNG because EMP starts to decompose at higher temperature than LNG and according to the values of the activation energy of the first decomposition step of both drugs, EMP has higher activation energy values than those of LNG.

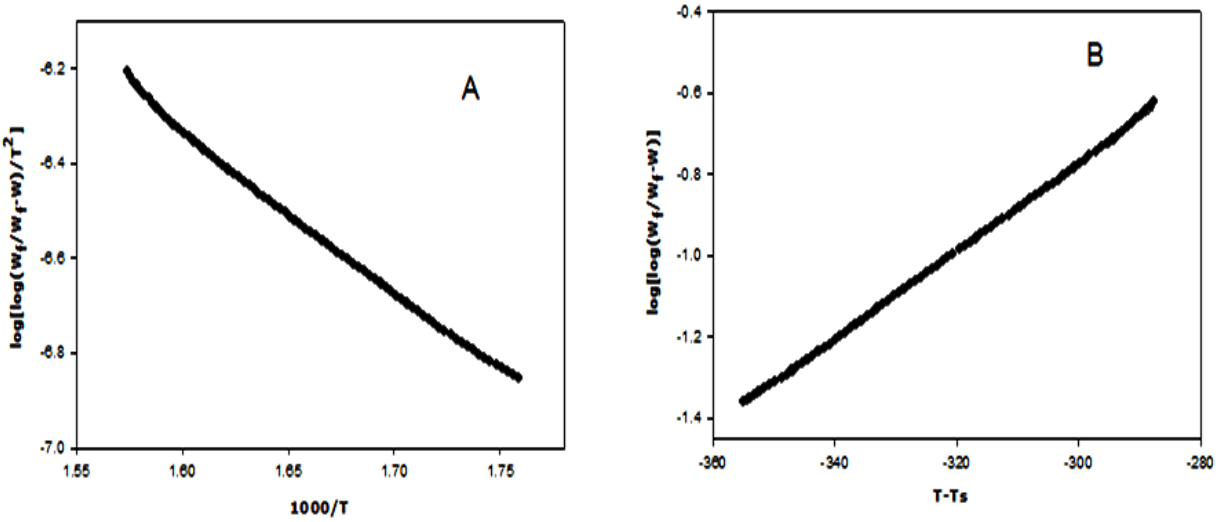

Figure 4: Coats-Redfern (A) and Horowitz-Metzger (B) plots of the first decomposition step of LNG

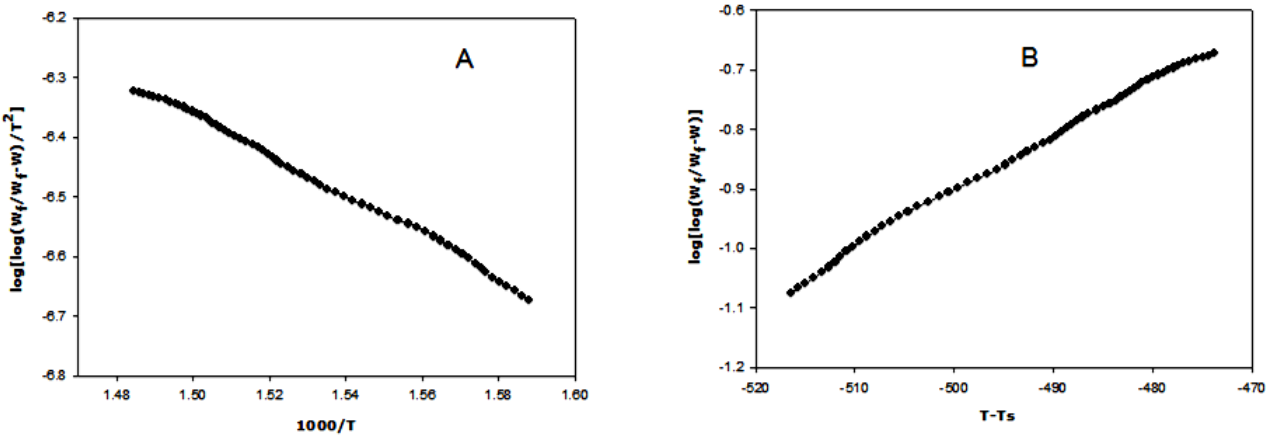

Figure 5: Coats-Redfern (A) and Horowitz-Metzger (B) plots of the second decomposition step of LNG

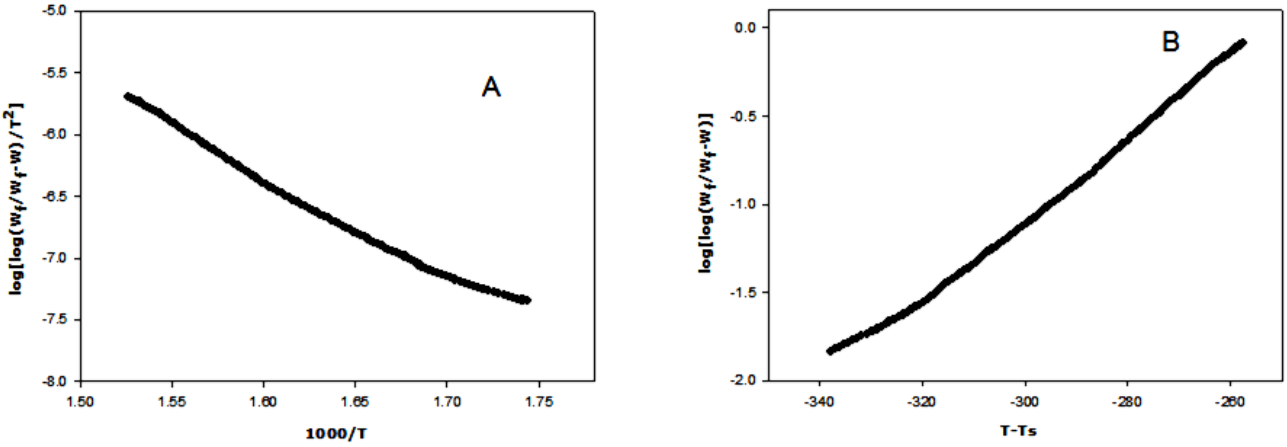

Figure 6: Coats-Redfern (A) and Horowitz-Metzger (B) plots of the first decomposition step of EMP

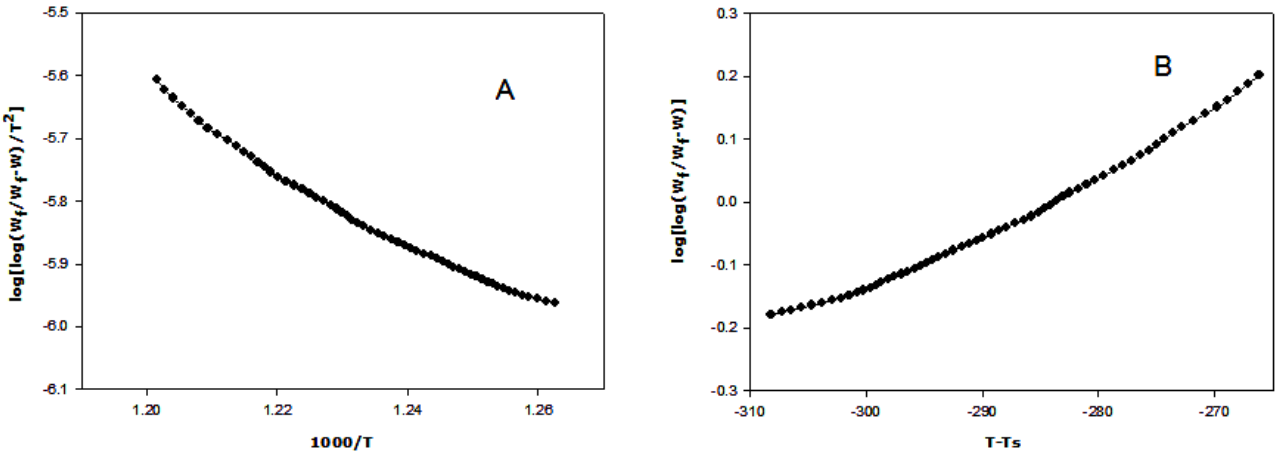

Figure 7: Coats-Redfern (A) and Horowitz-Metzger (B) plots of the second decomposition step of EMP

% Purity of LNG and EMP

Figure 8 shows the DSC curves of LNG, and EMP, figure 8A (DSC curve of LNG) exhibits an endothermic peak at 203.73^{o}C (melting of LNG) which appear at 205.63^{o}C in the DTA curve of LNG. Figure 8B (DSC curve of EMP) displays an endothermic peak at 152.62^{o}C (melting of EMP) which appear at 154.47^{o}C in the DTA curve of EMP. Van't Hoff equation: $T_f = T_0 - [(R\ T_0^2\ x/\Delta H_f)$. 1/F [74] is used to calculate the purity value of drugs, where T_f is the melting temperature of the sample, T_0 is the melting point of pure substance in Kelvin (K), R is the gas constant, ΔH_f is the heat of fusion, F is the fraction of sample melted at T_f, and x is mole fraction of impurities in the sample. The purity values of LNG and EMP are found to be 99.88% and 99.92%, respectively.

Table 2 introduce the purity values of LNG and EMP using DSC method and reported methods[6, 31], table 2 also shows the melting point values of LNG and EMP obtained from DTA and DSC curves, literature[75, 76] and using melting point apparatus. We note that the purity values are very similar using these methods, and the melting point values are comparable and close to each other for the two drugs. From the obtained results, we conclude that thermal analysis can be used as a quality control tool to determine melting point and purity of the studied drugs.

Table 2 Melting point and degree of purity of LNG and EMP and endothermic and exothermic peaks of inactive ingredients

Drug	Melting point (^{o}C)				Loss on drying%		Ash content%		Degree of purity%	
	DTA method	Melting point apparatus	DSC Method	Reported data [75,76]	TGA method	Reported data	TGA method	Reported data	DSC method	Reported data [14.31]
LNG	205.63	204	203.73	202 [75]	0.9	0.24	0	0.05	99.95	99.40[14]
EMP	154.47	153	152.62	(151-153) [76]	0.03	0.31	0	0.01	99.97	99.20[31]

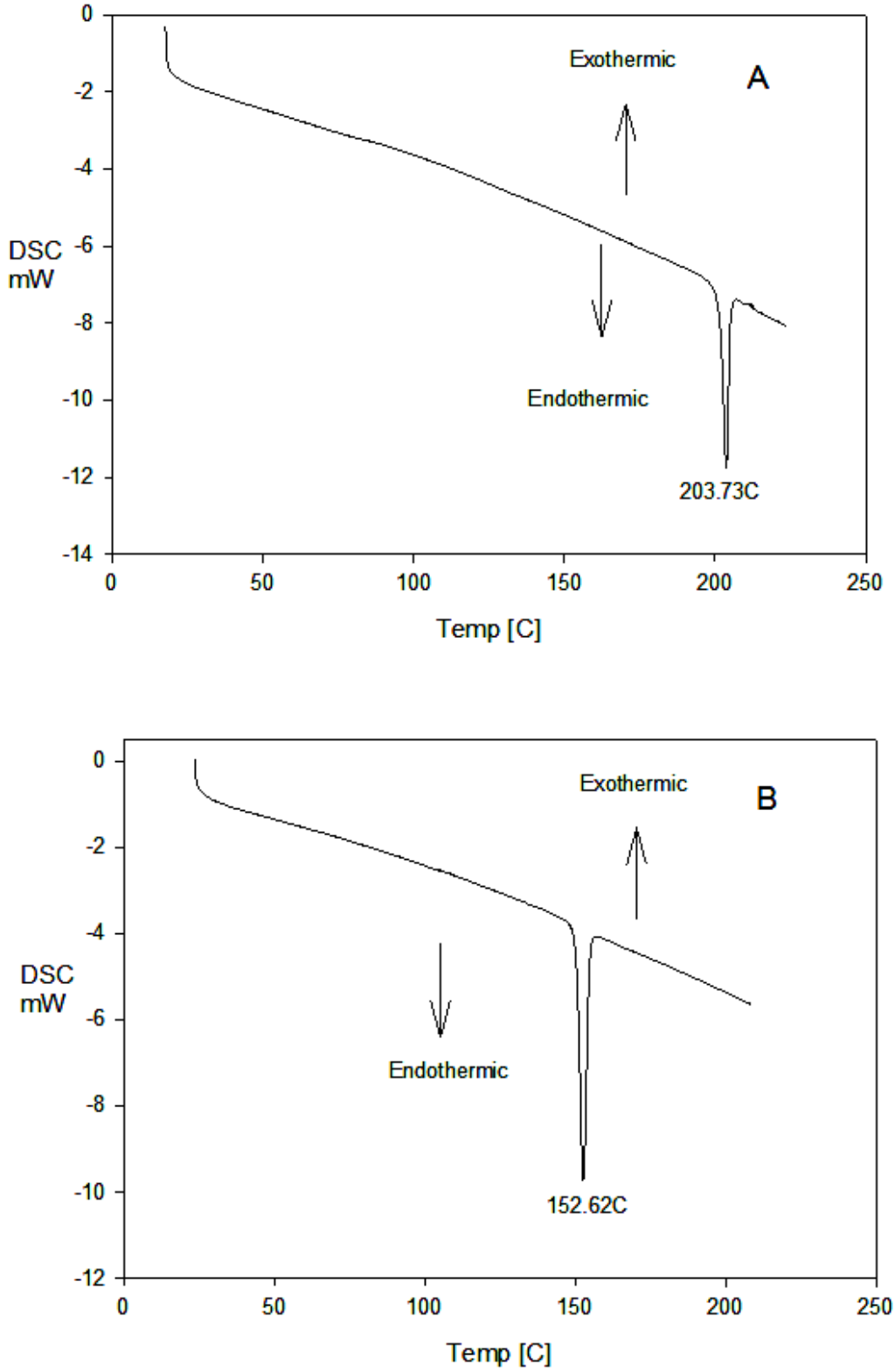

Figure 8: DSC curves of LNG (A) and EMP (B) at heating rate of 10^{o}C/min

Infra-red (IR) spectra

The IR spectrum of LNG (Figure 9 (A)) shows the presence of absorption bands at 3428 cm^{-1} (NH_2 stretching), 2932 and 2852 cm^{-1} (aliphatic C-H stretching), 1655 cm^{-1} (C=O stretching), 1517 cm^{-1} (C=N stretching), 1286 cm^{-1} (C-N stretching) and 763 cm^{-1} (C≡C bending).

The IR spectrum of EMP (Figure 10 (B)) shows the presence of absorption bands at 3423 cm^{-1} (O-H stretching), 3250 and 3058 cm^{-1} (aromatic C-H stretching), 2926 and 2866 cm^{-1} (aliphatic C-H stretching) and 1061 cm^{-1} (C-O stretching) [77,78].

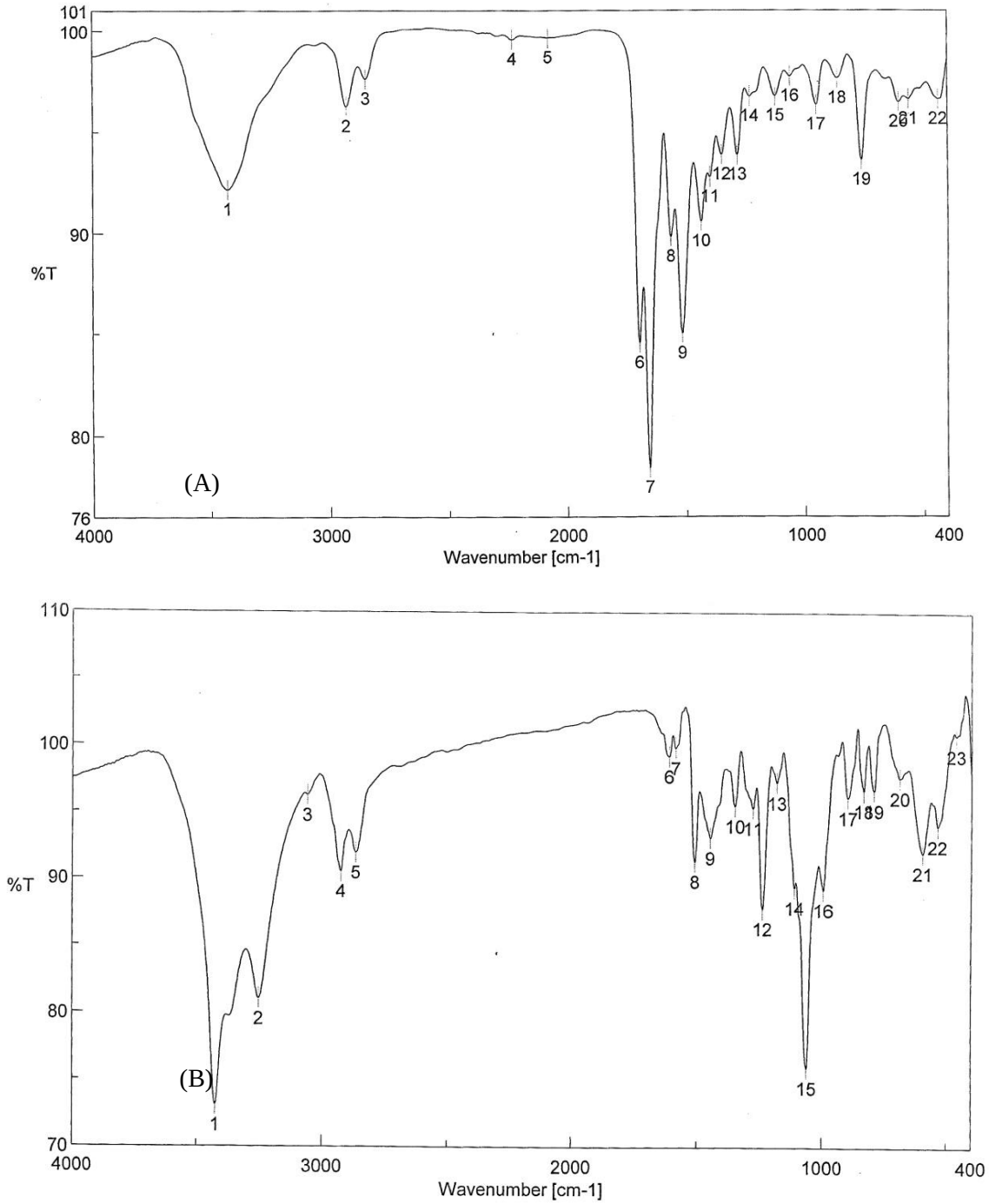

Figure 9: FTIR spectra of LNG (A) and EMP (B)

Conclusion

In this work thermal analysis and differential scanning calorimetry (DSC) techniques are used for the screening or testing the stability of LNG and EMP. The studied drugs are characterized by having main decomposition reaction which consists of two stages. Besides stability studies, thermal analysis is of value for determining melting temperature and water content. The melting points obtained by DSC reveal the precision of the technique in yielding this thermal parameter; this justifies the use of DSC as a routine technique for the identification of compounds. Comparison of the data obtained in this work exhibit the importance of the thermal analysis and DSC techniques for the quality control of bioactive drugs; it provides a rapid method for purity determination attending a value between 98% and 102% which is in agreement with the reported methods. The applied analytical methods in this work are characterized by being economic, fast and simple techniques; these are the reasons behind the even growing importance of thermal analysis in the quality control of the studied drugs.

Acknowledgements

The authors extend their appreciation to the National Organization for Drug Control and Research for providing the possibilities and devices to accomplish this work.

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