Thermal Characterization of Leflunomide

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Abstract: Purpose: The aim of the present work is to study the thermal behavior of leflunomide drug substance and drug product. **Methods:** Differential Thermal Analysis (DTA), Differential Scanning Calorimetry (DSC) and thermogravimetric analysis (TG/DTG) determined the raw material's purity, kinetic parameters, thermal behavior and melting characteristics. The thermogravimetric data allow determination of kinetic and thermodynamic parameters: Activation energy (E_a), activation entropy (AS^*) and reaction order (n) which calculated from TG-curves at different heating rates using different kinetic models: The relative thermal stability of the drug has been evaluated on the basis of the kinetic and thermodynamic parameters. Also, the purity of the drug is determined by Differential Scanning Calorimetry (DSC) technique. **Results:** The value of purity found is $99.997 \pm 0.09\%$. Quality control parameters obtained from the thermal analysis of leflunomide are melting point 164.42°C , water content 0% and ash content 0%. **Conclusion:** The simplicity, speed and low operational costs of thermal analysis justify its application in quality control of pharmaceutical drugs. DSC data indicated that the degree of purity of leflunomide is similar to that found by official methods.

Key words: Leflunomide, DSC, TG, DTG, DTA, vamid tablet, quality control

INTRODUCTION

Leflunomide is a pyrimidine synthesis inhibitor belonging to the DMAR (disease-modifying antirheumatic drug) class of drugs, which are chemically and pharmacologically very heterogeneous. Leflunomide, 5-methy-N-[4-(trifluoromethyl) phenyl]-4-isoxazolecarboxamide (Budavari *et al.*, 2006; USP, 2009).

Leflunomide has immunosuppressant properties. It is used as a disease-modifying anti rheumatic drug in the treatment of active rheumatoid arthritis and also in the treatment of active psoriatic arthritis. It is being investigated in the management of various solid neoplasm (Martindale, 2009). It is well absorbed after oral administration. Leflunomide is a prodrug that is

rapidly metabolized in the liver via opening of the isoxazole ring to the active metabolite and then excreted urine and feces (Manna *et al.*, 1999; Martindale, 2009).

Various methods have been mentioned in literature for the analysis of leflunomide in pure form, in pharmaceutical formulations and in biological fluids such as spectrophotometric method (Yenicelia *et al.*, 2005) and chromatographic methods (Dias *et al.*, 1995); Fairbanks *et al.*, 1999; Schmidt *et al.*, 2003; Roon *et al.*, 2004; Chan *et al.*, 2004; Yenicelia *et al.*, 2005).

The thermoanalysis methods refer to a group of techniques in which a physical property of a substance and/or its reaction products is measured as a function of a controlled temperature program (Giron, 2002). The most widely used techniques are Differential Thermal Analysis (DTA), Differential Scanning Calorimetry (DSC) and thermogravimetry/derivative thermogravimetry (TG/DTG). These techniques are widely used in the pharmaceutical sciences for the characterization of solid drugs and excipients. These techniques are established for quality control, preformulation, stability, drug-excipients interactions, polymorphism and purity studies of raw materials and pharmaceutical products (Mashru *et al.*, 2005; Xu *et al.*, 2006; Vega *et al.*, 2006; Saliva *et al.*, 2007; Ning and Xu, 2010).

MATERIALS AND METHODS

Materials: Leflunomide, raw material and Vamid® tablets were provided from ALKAN PHARMA, S.A.E., Co.S.A.E. 6th of October city, EGYPT. Each tablet is labeled to contain 100 mg of Leflunomide with batch No. 008.

Methods

Thermogravimetric analysis (TG) and differential thermal analysis (DTA): TG/DTG and DTA studies were made with simultaneous DTA-TG-Shimadzu DTG-60 H thermal analyzer. Approximately 5 mg of samples, using a platinum pan, were carried out from ambient temperature to 800°C in dynamic nitrogen atmosphere with the flow rate of 30 mL min⁻¹ and heating rate of 10°C min⁻¹.

Differential scanning calorimetry (DSC): DSC curves were measured on Shimadzu DSC-50 cell. Approximately 2 mg of samples were mass out and placed in a sealed aluminum pan. An empty aluminum pan was used as a reference. The purity determination was performed using heating rate of 2°C min⁻¹ in the temperature range from 25 to 185°C in nitrogen atmosphere with flow rate of 30 mL min⁻¹. The T_0 , T_g and ΔH_{fusion} were calculated in three replicates by Shimadzu TASYs software. DSC equipment was preliminary calibrated with standard of indium.

Kinetics: Solid state reactions include phase transition such as melting, evaporation and sublimation, as well as decomposition reactions resulting in the production of different compounds. Thermal analysis can be used to monitor these reactions by determining the rate of mass loss as the sample undergoes the process, using simultaneous TGA-DTA with a rising temperature program. The specific rate constant and the kinetic mechanism are determined with experimental data obtained from the TGA plot % mass loss versus time or temperature and the derivative analysis (DTG) plot of rate of mass loss (dm/dt) versus time or temperature. The specific rate constants are determined by Eq. 1:

$$K = [(\alpha/\beta) f(\alpha)] \quad (1)$$

where, α is the fraction reacted and β is heating rate in degrees/sec. the term $f(\alpha)$ can be obtained from tables of different mechanisms. For the first order mechanism, it is equal to $(1-\alpha)$. The Arrhenius equation is used to calculate the activation energy:

$$\ln K = \ln A - E_a/RT \quad (2)$$

where, K is the specific rate constant, E_a is the activation energy, R is gas constant, A is the pre-exponential term and T is the temperature in Kelvin. A plot of $\ln K$ versus $1/T$ is linear with a slope equal to E_a/R and an intercept equal to A .

RESULTS AND DISCUSSION

TG/DTG and DTA curves of the leflunomide are seen in Fig. 1 and 2 TG/DTG curves show two thermal decomposition steps. The first step shows a large mass loss of 91.63% (found 89.5%) in the range of 170-262.59°C. In addition a mass loss of 8.4% (found 10.3%) is observed in the interval 262- 400°C as shown in Table 1. The first thermal decomposition step is suggested to be due to the elimination of (C₁₁H₉F₃N₂O). The second thermal decomposition step is may be due to the elimination of C = O group. The decomposition is confirmed by DTA curve that indicates two endothermic peaks in temperature range (165.8-176.15°C) and (232.23-250.8°C) as shown in Table 2. DTA curve shows an endothermic peak at 168.4°C (reported range 164-168°C). This event may be corresponding to the melting of the compound. DSC gives a value of 164.4°C for melting point.

Fig. 1: TG/DTG curves of leflunomide

Table 1: Thermo gravimetric reaction

First reaction				Second reaction			
Start (°C)	End (°C)	wt. loss (%)	DTG Peak	Start (°C)	End (°C)	wt. loss (%)	DTG Peak
170.45	262.59	91.6	236.34	262	400	8.5	299

Table 2: DTA reactions

First reaction				Second reaction			
Start (°C)	End (°C)	wt. loss (%)	DTG Peak	Start (°C)	End (°C)	wt. loss (%)	DTG Peak
Endo	165.8	168.43	176.15	Endo	232.23	243.15	250.85

Table 3: Thermodynamic parameters for the thermal decomposition of leflunomide

Methods	Temperature range (°C)	Thermodynamic parameters				
		E_a (kJ mol ⁻¹)	A (S ⁻¹)	ΔS (kJ mol ⁻¹ . K)	ΔH (kJ mol ⁻¹)	ΔG (kJ mol ⁻¹)
Coats -Redfern	170.5-262.6	111.835	6.1×10^{11}	-22.478	108.198	118.024
Horowitz and Metzger	170.5-262.6	97.317	2.61×10^{11}	-29.54	93.682	106.595

Fig. 2: DTA curve of leflunomide

Fig. 3: TG curves obtained at 5, 7.5, 10, 12.5, 15°C min⁻¹ in N₂ (30 mL min⁻¹)

Non-isothermal kinetic study

Ozawa's method: Non-isothermal kinetic study is performed by the application of Ozawa's method (Ozawa, 2000). In dynamic experiments heating rates of 5, 7.5, 10, 12.5, 15°C min⁻¹ were used to target temperature 800°C as shown in Fig. 3, in order to determine the activation energy (E), Arrhenius frequency factor (Z) and order of reaction (n). The equipment was preliminarily calibrated with standard reference of calcium oxalate. The effect of heating rates were described to drug captopril (Huang *et al.*, 2001). The activation energy was obtained from a plot of logarithms of heating rates (A) as a function of the inverse of temperature (1/T) for a constant G (x), where G (x) is the integrated form of the conversion dependence function, f (x) (Fig. 4). The kinetics parameters obtained were activation energy (E) in kJ mol⁻¹, frequency factor (Z) min⁻¹ and zero order reaction (n = 0).

Fig. 4: Integrated form of constant G (x) and the convention dependence function

Horowitz and Metzger method: For the first order kinetic process, the Horowitz-Metzger equation (Horowitz and Metzger, 1963).

Coats-Redfern method: For the first order kinetic process, the activation energy (E_a) in J/mole could be calculated from Coats-Redfern equation (Coats and Redfern, 1964). The entropy ΔS^* , enthalpy ΔH^* and free energy ΔG^* of activation were calculated using the following equations:

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

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

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

where, k and h were the boltzman and plank constant, respectively. So, the calculated values of E*, ΔS^* , ΔH^* and ΔG^* could be obtained as shown in Table 3.

Determination of purity: The determination of purity is based on the assumption that an impurity will depress the melting point of a pure material whose melting is characterized by a melting point (T₀) and an enthalpy of fusion (ΔH_f). The melting transitions of a pure, 100% crystalline material should be infinitely sharp, but impurities or defects in the crystal structure will broaden the melting range and lower the final melting point to a temperature lower than T₀ (Hatakeyama and Liu, 1998). Purity determination is officially listed in United

Fig. 5: DSC curve obtained at heating rate of $2^{\circ}\text{C min}^{-1}$ in N_2 (30 mL min^{-1}) of leflunomide

Table 4: Melting points and degrees of purity of the drug

Melting point				Degree of purity (%)	
DTA method	Melting* point	DSC Method	Literature	DSC Method	Official*** Method
168.4	164	164.42	164-168**	99.997%	99.89%

*Mettler FP 80, central processor, ** USP 32, ***HPLC

Table 5: Quality control parameters obtained from the thermal analysis of leflunomide compared with reported method (USP, 2009)

Parameters	Thermal analysis	Reported method
Melting point ($164-168^{\circ}\text{C}$)	164.42°C	164°C
Loss on drying (NMT 0.5%)	Zero %	0.09 %
Ash content (NMT 0.1%)	Zero %	Zero %

Instruments: melting point (Mettler FP 80, central processor), (Precisa XM 60, swiss made) for loss on drying and muffle furnace (Vulcan A-550) for ash content

States Pharmacopoeia in general chapter on thermal analysis (USP, 2009). The effect of an impurity on T_0 of Leflunomide was determined by DSC method basing on the Van't Hoff equation:

$$\frac{T_s - T_0}{\Delta H_f} = \frac{RT_s^2 \times 1/F}{\Delta H_f} \quad (6)$$

where, T_s is the sample temperature at equilibrium (K), T_0 is the melting point of the pure component (K), R is the gas constant, X is the concentration of impurity (mole fraction) and F is the fraction molten at T_s .

The obtained DSC curve is shown in Fig. 5, which exhibits the endothermic event corresponding to leflunomide's melting point ($\Delta H_f = 105.90$). The value of purity found was 99.997% confirming low impurity content, also data was compared with official method in Table 4.

Application of thermal analysis to quality control of leflunomide: Thermal analysis is used as an alternative technique for laboratory-screen tests (long-term tests),

Fig. 6: Thermal analysis (TG, DTG and DTA curves) of vamid tablet

Fig. 7: TG thermogram of leflunomide alone (—) and with different excipients, Lactose monohydrate (----), Mg stearate (····) and croscopolvidone (-.-.-)

for the determination of different quality parameters such as, melting point, water content and ash content. No significant difference was observed between the obtained results when compared with reported method as shown in Table 5.

Application of thermal analysis to vamid tablet: DTA, DTG and TG curves of leflunomide formulation are seen in Fig. 6. The thermal degradation occurs in three steps and the onset and endest temperature for degradation was shifted to lower temperature. DTA evidence an endothermic peak corresponding to leflunomide melting point in temperature range of 150 to 160°C followed by thermal decomposition. This is confirmed by TG curve that indicate mass loss event. It was observed that the drugs melting event occurs with mass loss, suggesting an interaction, but not necessary corresponding to incompatibility. In fact a similar effect was observed for other drug excipients mixtures and was attributed to drug dissolution in the melted excipients (Cides *et al.*, 2006).

Fig. 8: DSC curve obtained at heating rate of $2^{\circ}\text{C min}^{-1}$ in N_2 (30 mL min^{-1}) of leflunomide tablet

Based on the results of TG, majority of the excipients (mg stearate, lactose monohydrate and croscopolvidone) were found to be compatible with leflunomide as shown in Fig. 7. The drug was found to be stable up to 200°C in the presence of excipients.

Also the DSC curve of leflunomide tablet (Fig. 8) shows an endothermic peak at 167°C corresponding to melting point of leflunomide's melting point.

CONCLUSION

In the development of solid pharmaceutical forms, quality control, stability and compatibility studies are important parameters to ensure the final product quality. Thermoanalysis is a routine method current applied in pharmaceutical industry.

DSC provides a rapid method for purity determination, attending value between 98 and 102%, which is in agreement with the official published method. The decomposition process occurs in constant rate and is independent of the concentration of the reactants. When the limiting reactant is completely consumed the reaction abrupt stops. The excipients can produce a different environment in which the behavior of the drug is modified but they are still compatible with the drug.

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