



*Dedicated to Professor Victor-Emanuel Sahini  
on the occasion of his 85<sup>th</sup> anniversary*

## HYPHENATED TECHNIQUES USED BY EVALUATION OF THE THERMAL BEHAVIOUR OF GLYCYRRHETINIC ACID

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Glycyrrhetic acid is a pentacyclic triterpenoid derivative, its biological activity being studied thoroughly even if the reason for its wide spectrum of action is not fully understood. The aim of this work is to study the thermal behavior of glycyrrhetic acid, taking into account the presence of thermally sensitive functions (hydroxyl, carboxyl, ketone) in the molecule. After melting at 296 °C, a clear thermooxidative degradation with a maximum rate at 380 °C takes place. A comparison of FTIR-UATR spectra of the initial sample, respectively the remainder by 500 °C, together with EGA, allowed some comments on the reaction mechanism. A kinetic analysis using non-isothermal data was performed in order to obtain a set of believable kinetic parameters. The data processing strategy uses the Friedman's, Flynn-Wall-Ozawa and modified NPK methods.

### INTRODUCTION

Glycyrrhetic acid, (I), (see Scheme 1) is a pentacyclic triterpenoid derivative of the beta-amyrin type (IUPAC Name: 10-hydroxy-2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo-3,4,5,6,6a, 7,8,8a,10,11,12,14b-dodecahydro-1H-picene-2-carboxylic acid). The average molecular mass is 471 a.m.u. It is obtained by hydrolysis of glycyrrhizic acid, a triterpenoid saponin glycoside extracted (ethanol/water, 30/70, v/v) from roots and stolons of some Glycyrrhiza species (Fabaceae).<sup>1</sup>

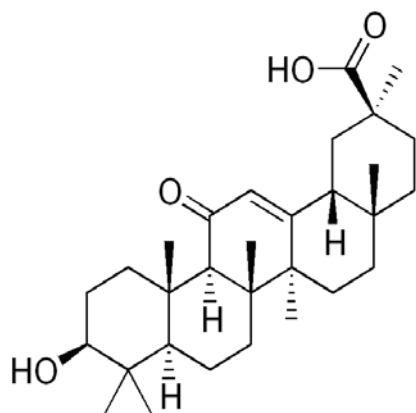
The biological activity of (I) has been studied thoroughly, antiviral, anticariogenic, antiangiogenic, antiulcerogenic, hepatoprotective, even if the reason for its wide spectrum of action is not fully

understood.<sup>2</sup> Widely, (I) is used in cosmetic industry, especially against hyperpigmentation, respectively UV-B-induced pigmentation and erythema.<sup>3,4</sup> The Cosmetic Ingredient Review Expert Panel has decided that (I) is safe in the current practices of use and concentrations.<sup>5</sup>

The thermal behavior of (I) is important from the following points of view: a) technological, because by hydrolysis, extraction and especially drying the thermal treatment can induce some decompositions; b) biochemical, due to the thermally sensitivity (inhibition) of the tyrosinase activity respectively of other enzymes involved in steroids metabolism; c) fundamental, due to the presence of thermally sensitive functions (hydroxyl, carbonyl, ketone) in a rather complex molecular architecture.

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The aim of this paper is to study the thermal behavior of (I) using hyphenated techniques and an appropriate kinetic analysis.



Scheme 1 – Glycyrrhetic acid.

## RESULTS AND DISCUSSION

### Thermoanalytical data

According to the diagram in Fig. 1, the following thermal induced phenomena were observed in air atmosphere: a) melting at 297.5°C (endothermic peak without mass loss); the melting heat was 26.0 kJ·mol<sup>-1</sup>; b) thermooxidative degradation between 320 and 400°C with a single DTG maximum at 387°C; the exothermic effect is -230.9 kJ·mol<sup>-1</sup>, therefore the DTA/HF maximum is ill-timed with 5-7°C in respect of the DTG one; c) the mass loss by this thermooxidation is over 80%, indicating a high extent of the degradation; d) after this relative narrow interval of 80°C, a combustion process

occurs, at a temperature over 400°C; the DTA maximum of this step is at 431°C and the exothermic effect is -110.4 kJ·mol<sup>-1</sup>. This char combustion is behind the present topics, so that the drawing up was stopped at 500°C.

In nitrogen atmosphere, as expected, the thermal behavior is different (see Fig. 2.)

a) the melting point at 301°C is preserved, with a melting heat of 26 kJ·mol<sup>-1</sup>; b) the mass loss is total and takes place between 350°C and 450°C with a DTG maximum at 404°C, equal with the DTA maximum; the endothermic thermal effect is 42.7 kJ·mol<sup>-1</sup> (liquid/gas phase transformation process).

### EGA data

The GS profiles of the EG in air, respectively nitrogen are presented in Fig. 3. For the correspondence between the abscisa of TG/DTG diagram (Temperature units) respectively of GS profile (time units) the heating rate of 20°C·min<sup>-1</sup> has to be taken into account.

By the maximum of these GS diagrams the FTIR spectra in Figs. 4 and 5 were obtained for the EG. In air, the main components of EG at the maximum reaction rate being water and carbon dioxide, it means that a total oxidation takes place. This is in agreement with the high exothermic effect (-230.9 kJ·mol<sup>-1</sup>).

In nitrogen atmosphere the EG was identified as glycyrrhetic acid vapor. Taking into account also the total mass loss and the relative low endothermic effect (42.7 kJ·mol<sup>-1</sup>), we suppose an evaporation process.

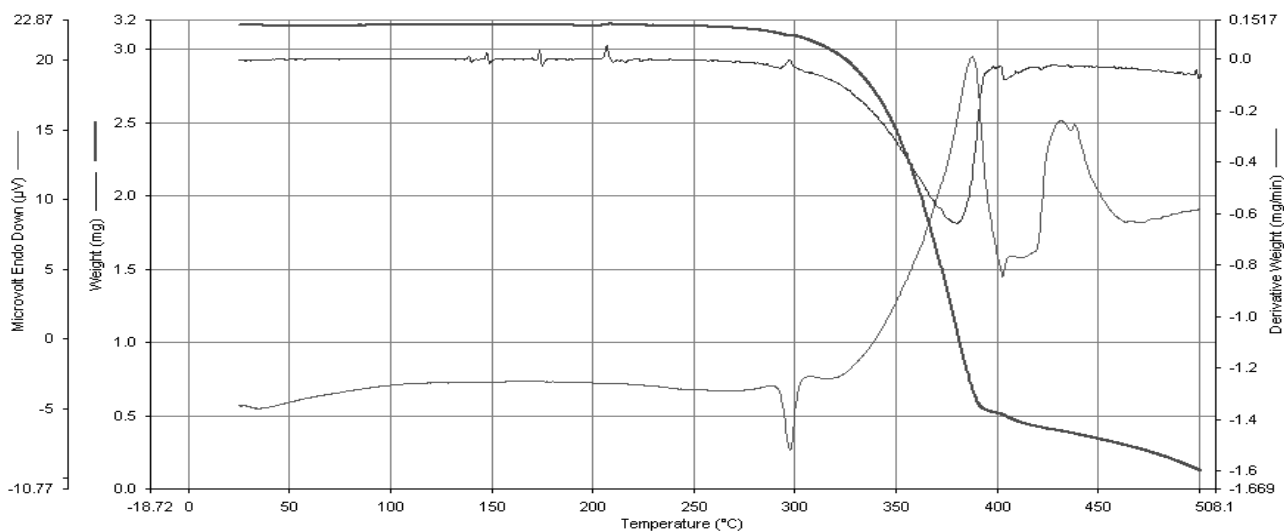


Fig. 1 – TG/DTG/Heat Flow diagram obtained in dynamic air at 10 °C·min<sup>-1</sup>.

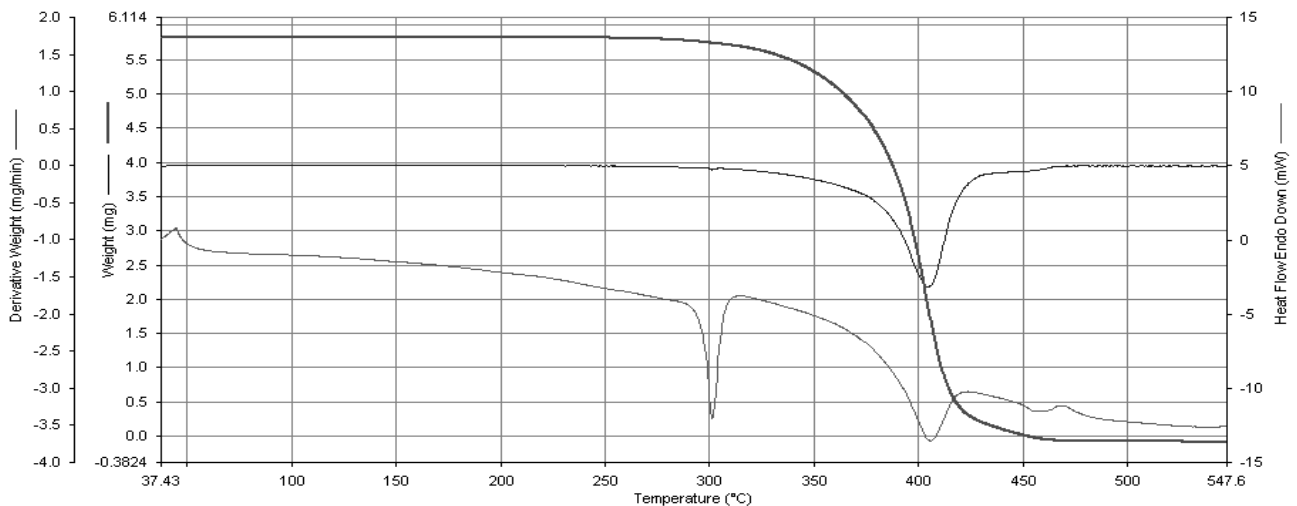


Fig. 2 – TG/DTG/Heat Flow diagram obtained in dynamic nitrogen at  $10^{\circ}\text{C}\cdot\text{min}^{-1}$ .

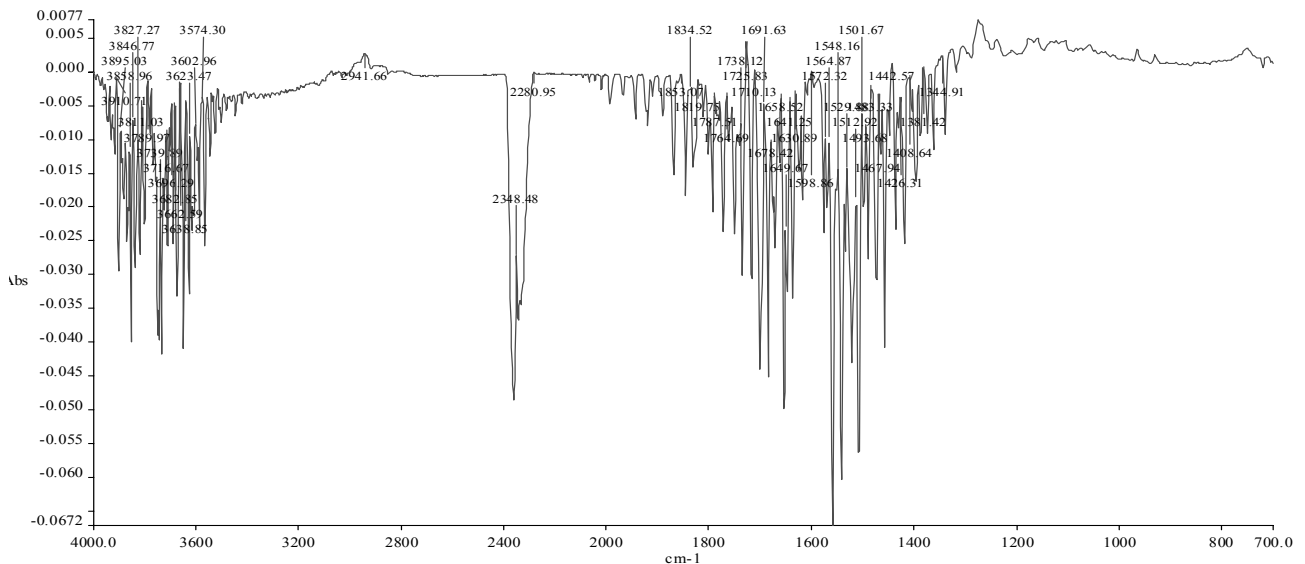


Fig. 3 – FT-IR spectrum of Evolved gases at  $380^{\circ}\text{C}$  in air atmosphere; the main present compounds are water vapor and carbon dioxide.

### UATR data

The FTIR spectra of (I) and of the crucible residue after the thermal treatment in air until  $400^{\circ}\text{C}$  were obtained by the UATR technique and the main data are presented in Table 1.

By inspecting these data the disappearance of the peaks corresponding to thermal and oxidation sensitive functions was observed. A rather unsaturated residue was later oxidated (an exothermic effect of  $-110.4\text{ kJ}\cdot\text{mol}^{-1}$ ).

According to the former observations and discussions we suggest the following thermally induced processes: (i) in air: melting  $\rightarrow$  thermooxidative degradation in melted phase  $\rightarrow$

oxidation of an unsaturated/aromatic residue; (ii) in nitrogen: melting  $\rightarrow$  evaporation.

We uphold the hypothesis of thermooxidation in melted phase due to the presence of a residue after heating up to  $400^{\circ}\text{C}$ . By the following kinetic analysis we bring more details about this step.

### FTIR data

The FTIR spectrum of the Evolved Gases, in air, drawn at the DTG maximum of the thermooxidative step (see Fig. 3) indicates that the main reaction products were carbon dioxide and water, i.e. the oxidation in gas phase is complete.

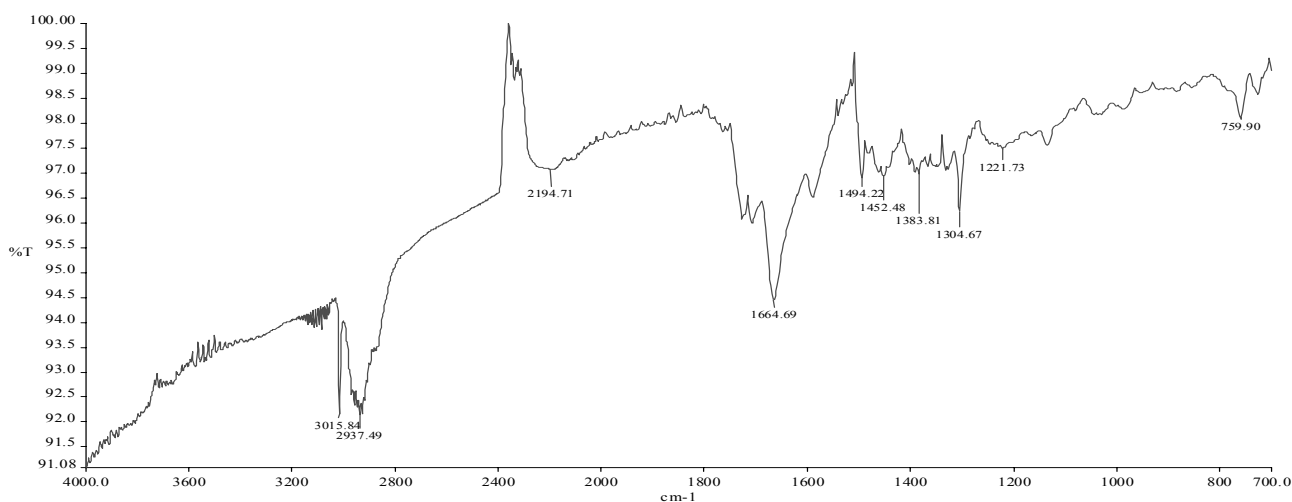


Fig. 4 – FT-IR spectrum of Evolved Gases at 400°C in nitrogen atmosphere – glycyrrhetic acid is the identified compound.

Table 1

IR data of I and the corresponding char at 500°C (U-ATR)

Glycyrrhetic acid		Char	
Wave number, cm <sup>-1</sup>	Assignment	Wave number, cm <sup>-1</sup>	Assignment
3434.9, 2926.4	intramolecular OH	2235	R <sub>1</sub> C≡CR <sub>2</sub>
2147-2191.8	intramolecular OH	2158	RC≡CH
1955.9-1987.3	arom.conj.	1999,1957	allenes
1702.5	carboxylic acid	1559	C=C, conj.
1663.9	carboxylate ion		
1449.6	OH acid		
1366, 1342.7	OH alcohols		
1308, 1213, 1177	ketones		
1026, 991	primary alcohols		

The FTIR spectrum obtained by means of the U-ATR technique (see Table 1) confirms the deep degradation of the remaining char. The aromatization/unsaturation was the trend of the molecular transformations.

According to the spectrum in Fig. 4 in nitrogen atmosphere, the Evolved Gas consists in Glycyrrhetic Acid vapors. This is an argument for considering that the total mass loss is due to an evaporation/boiling step.

The differences of the thermal behavior in air respectively in nitrogen are worthy of discussions. The differences between melting points (m.p.) is not essential, taking into account that under non-isothermal conditions there are dynamic conditions for m.p. determinations.

Regarding the oxidation, respectively the combustion steps, the high negative values of the thermal effect were expected. The exothermic effect of the first step is twice in comparison with the second one.

In nitrogen, after melting, an evaporation/boiling process takes place. This is the reason for the total mass loss with an endothermic effect ten times lower than the thermal effect of the oxidative degradation.

### Kinetic analysis

In order to acquire a better understanding of the thermooxidative process, a kinetic analysis was performed on the TG/DTG data between 320 and 400°C. In nitrogen atmosphere the thermal induced phenomenon being evaporation, a kinetic analysis would be irrelevant. The ICTAC 2000 Protocol recommendations were taken into account,<sup>6</sup> *i.e.* all the used methods were isoconversional.

1. The differential method of Friedman (FR):<sup>7</sup> Under non-isothermal conditions and accepting an Arrhenius type temperature dependence, the reaction rate is:

$$\left(\beta \frac{d\alpha}{dT}\right)_\alpha = f(\alpha)A \exp\left(-\frac{E}{RT}\right) \quad (1)$$

where:  $\alpha$  is the conversion degree,  $\beta$ -the heating rate,  $A$  and  $E$ -the preexponential factor, respectively the activation energy,  $(d\alpha/dT)$  is the direct available DTG data and  $f(\alpha)$  is the conversion function.

By linearization in the form

$$\ln\left(\beta \frac{d\alpha}{dT}\right)_\alpha = \ln[A \cdot f(\alpha)] - \frac{E}{RT} \quad (2)$$

the value of  $E$  will be determined. Because  $f(\alpha)$  can't be explained, this strategy is a "model free" one.

Due to its simplicity and independence in respect of the kinetic model, the FR method is recommended for the beginning of all kinetic analyses strategies.

According to the data in Fig. 5, the variations of  $E$  vs.  $\alpha$  don't exceed 10%, this being a sign of a simple process.

The mean value is  $102.5 \pm 1.5 \text{ kJ}\cdot\text{mol}^{-1}$ .

2. The integral method of Flynn-Wall<sup>8</sup> and Ozawa<sup>9</sup>(FWO):

By integration of eq. (2), it becomes:

$$\ln \beta = \ln \frac{A}{R \cdot g(\alpha)} - 5.331 - 1.052 \cdot \frac{E}{RT} \quad (3)$$

where:  $g(\alpha) = \int \frac{d\alpha}{f(\alpha)}$  is the integral conversion function .

Considering the corresponding temperature  $T_i$  for different heating rates at the same conversion  $\alpha_i$  (isoconversional conditions) a plot of  $\ln\beta$  vs.  $1/T_i$  gives a straight line from which slope the activation energy can be calculated.

According to this strategy, the data in Fig. 6 were obtained. Taking into account that FWO method, being an integral method, it expresses the "thermal history" of the sample, the  $E$  vs.  $\alpha$  variation is in the reasonable range of 10%. Also, because the "thermal events" takes place in the interval of 80 deg, these both ascertain are arguments for a simple process.

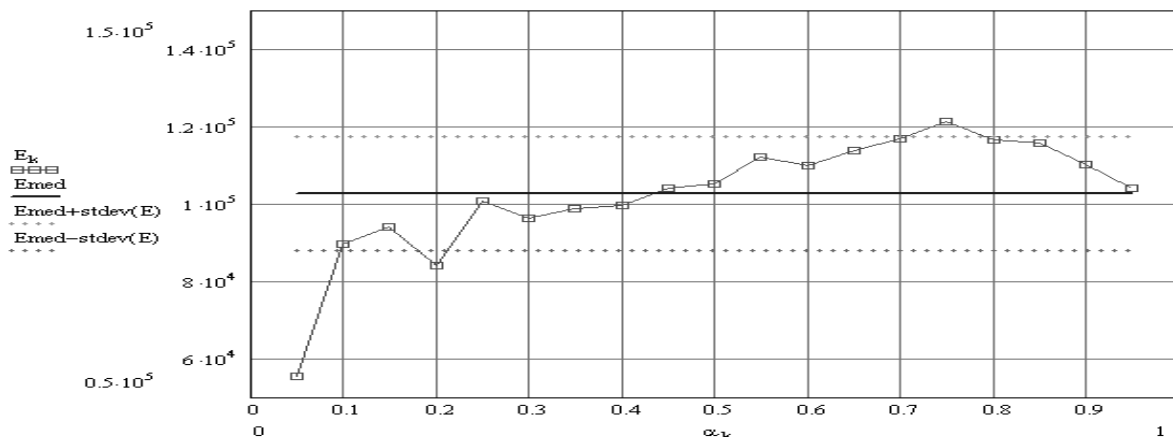


Fig. 5 – E vs.  $\alpha$  diagram according to Friedman's methods.

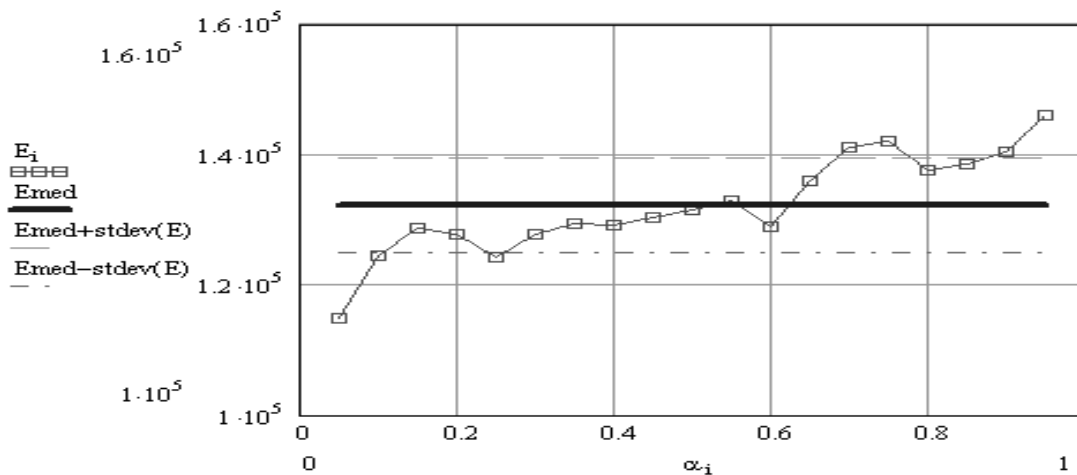


Fig. 6 – E vs.  $\alpha$  diagram according to FWO methods.

Table 2

The kinetic parameters according to NPK method

Process	$\lambda$ , %	A, s <sup>-1</sup>	E, kJ·mol <sup>-1</sup>	m (eq. 9)	n (eq. 9)	$\bar{E} = \sum \lambda_i \cdot E_i$ , kJ·mol <sup>-1</sup>
main	81.6	9.4·10 <sup>7</sup>	109.6	1	2/3	104.6
secondary	14.7	1.3·10 <sup>8</sup>	103.6	2/5	0	

The mean value is  $132.2 \pm 7.3$  kJ·mol<sup>-1</sup>.

3. The modified NPK method:<sup>10-14</sup>

The previous presented methods are “model free”, nothing being asserted about the conversion dependence of the reaction rate.

If we consider the reaction rate ( $r$ ) being represented by

$$r = f(T) \cdot g(\alpha) \quad (4)$$

then the experimental data are represented in a 3D space ( $r$ ,  $\alpha$ ,  $T$ ) and interpolated in order to generate a continuous surface corresponding to eq. (4).

This surface is discretised as a  $i$ - $j$  matrix  $M$

$$M = \{m_{ij}\} = \{f(T_i) \cdot g(\alpha_j)\} \quad (5)$$

Using the Singular Value Decomposition (SVD) algorithm,<sup>15</sup> the matrix  $M$  is decomposed as follows:

$$M = U(\text{diag } s) V^T \quad (6)$$

and the first columns of  $U$  and  $V$  matrix, respectively  $u_1$  and  $v_1$ , are analyzed for determining the kinetic model, i.e.:

$$u_1 = g(\alpha) \quad (7)$$

and a temperature dependence, i.e.:

$$v_1 = f(T) \quad (8)$$

For the temperature dependence, the classical Arrhenius equation is selected, whereas for the kinetic model we suggest the equation of Šestak-Berggren:<sup>16</sup>

$$g(\alpha) = \alpha^m (1-\alpha)^n \quad (9)$$

where  $\alpha^m$  describes the influence of physical phenomenon related with the presence of reaction product and  $(1-\alpha)^n$  describes the chemical phenomenon which implies the remainder reactant  $(1-\alpha)$ .

If the decomposition process is a result of two or more simultaneous steps, it means that  $r = \sum r_i$  and consequently the matrix  $M$  becomes:

$$M = \sum M_i \quad (10)$$

The contribution of each step to the observed process is expressed by the explained variance  $\lambda$ , so that  $\sum \lambda_i = 100\%$ .

If the decomposition is a process with two simultaneous reactions, the matrix  $M$  became:

$$M = M_1 + M_2 = u_1(\text{diag } s_1)v_1^T + u_2(\text{diag } s_2)v_2^T \quad (11)$$

where  $u_1$  and  $v_1$  correspond to the first columns of matrix  $U$  respectively  $V$  and  $u_2$  respectively  $v_2$  correspond to the secondary columns of matrix  $U$  respectively  $V$ .

The contribution of each step to the entire process being expressed by the explained variance  $\lambda$ , so that  $\lambda_1 + \lambda_2 = 100\%$ .

Noticeable is the ability of NPK method to separate a complex process in two (or more) elementary steps. Also, the SVD algorithm allows an objective analysis of temperature, respectively conversion dependence of the reaction rates.

The kinetic parameters according to this method are systematized in Table 2.

By inspecting these data the following remarks were drawn: a) the thermooxidation is rather a single step process ( $\lambda_1 > 80\%$ ), b) the value of the activation energy obtained by NPK methods is close to the main value obtained by Friedman's method, a supplementary argument for considering the thermooxidation a single step process, even if the kinetic model implies a function of both  $\alpha$  and  $(1-\alpha)$  respectively.

## EXPERIMENTAL

Glycyrrhetic acid, pharmaceutical grade (Sigma Aldrich), was used.

The experimental data were obtained by the following hyphenated techniques: TG-DTG-DTA, Evolved gas analysis (EGA), Universal Attenuated Transmittance Reflectance (UATR). These were possible by a physical and soft-ware coupling of two Perkin-Elmer devices: a Diamond thermobalance and a Spectrum 100 spectrometer.

The TG data were obtained in dynamic atmosphere (100 mL·min<sup>-1</sup>) and five heating rates: 7, 10, 12, 15 and 20 °C·min<sup>-1</sup>. The used gases were from Linde: synthetic air (80:20) and nitrogen, both of 5.0 purity. For the determination of the thermal effect the DTA data ( $\mu$ V) were converted into Heat Flow data (mW). So the peak area corresponds to the thermal effect (J·g<sup>-1</sup> or kJ·mol<sup>-1</sup>). The IR identification of the evolved gases was performed by the Sadtler Gas Vapor Library, using the FTIR spectra obtained by means of the gas chamber of Spectrum 100.

The EGA technique with a FTIR spectrometer allows us to collect the spectrum which is characterized by different wave numbers  $\nu_i$  and the corresponding extinction intensities  $I_i$ :

$$S = \begin{pmatrix} \nu_1 \nu_2 \dots \nu_n \\ I_1 I_2 \dots I_n \end{pmatrix} \quad (12)$$

The Root Mean Square is defined by

$$RMS = \left( \frac{1}{n} \sum_{i=1}^n I_i^2 \right)^{1/2} \quad (13)$$

Usually by EGA the Gramm-Schmidt (GS) profile is drawn up. By a thermal analysis under non-isothermal conditions with a constant heating rate, the GS profile is a RMS vs. time diagram by which the background spectrum was subtracted.

According to Lambert-Beer's law, for each peak, the extinction intensity  $I_i$  is proportional with the concentration of the component  $C_i$ . Consequently, if the decomposition reaction attains its maximum rate, the GS profile also attains a maximum. The FTIR spectrum at this time/temperature gives useful data on the EG composition, respectively on the decomposition process.

The UATR technique allows us to obtain the FTIR spectra of solid samples of both (I) and remainder char after the thermal degradation.

## CONCLUSIONS

A natural product with a rather complex structure was investigated in respect of its thermal behavior.

An hyphenated technique together with an advanced strategy of TG/DTG data processing allows some discussions on the thermooxidative degradation. This process depends rather on an integral conversion function.

The narrow values of the activation energy, obtained by at least two different data processing methods, together with an explicit form of the kinetic function are premises of a believable thermal behavior characterization.

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