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Stability of Diclofenac Sodium in the Inclusion Complex with β-Cyclodextrin in the Solid State

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Abstract

The aim of this study was to characterize the thermal stability of diclofenac sodium both alone and in the inclusion complex with β-cyclodextrin in the solid state, by determination of the number of the products of its decomposition, which were identified by GC–MS.

The molar ratio of diclofenac sodium in the inclusion complex with β-cyclodextrin was 1 : 1. The decomposition of diclofenac sodium both alone and in inclusion complex with β-cyclodextrin occurred according to the first-order reaction. The HPLC of the samples thermostated at 80°C gave five products of decomposition, which were identified by GC–MS.

Diclofenac sodium in the inclusion complex with β-cyclodextrin was more thermally stable. Thermal decomposition of diclofenac sodium leads to formation of five products, of which 4-chloro-10H-9-acridinone had not been reported previously in the literature.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac sodium, are often used in the treatment of rheumatic diseases (Brogden et al 1980; Orienti et al 1991; Kaiser & Pineda 1997). The mechanism of action of NSAIDs involves inhibition of prostaglandin cyclooxygenase necessary for metabolism of arachidonic acid (Van Bruchhausen et al 1993). NSAIDs can cause a number of undesirable effects such as ulcerogenic or in some cases hepatotoxic effects. Stability of these drugs is of utmost importance as the products of their decomposition may enhance the undesirable effects.

Recent literature has brought many reports on the physical and chemical properties of diclofenac sodium (Clarke 1986; Sato et al 1997), and its pharmacological effects (Sagara et al 1992). However, little information has been given on its stability. One of the methods used to increase the stability of a pharmaceutical is through the formation of its inclusion complex with a cyclodextrin (Szejtli 1988; Duchéne & Wouessidjewe 1990; Connors 1995).

The aim of this study was to characterize the thermal stability of diclofenac sodium both alone and in inclusion complex with β-cyclodextrin in the solid state, determining the number and identity of its decomposition products, as well as explaining the mechanism of thermal decomposition of this substance.

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Materials and Methods

Materials

β-Cyclodextrin was from Chinoin (Budapest, Hungary). Diclofenac sodium, acetonitrile and methanol (HPLC purity) were purchased from Sigma Chemical Co. (St Louis, MO). Deuterated dimethylsuphoxide (DMSO-d_6) (99·95%) was obtained from Merck KGaA (Darmstadt, Germany). All other reagents were of analytical grade.

Preparation of the diclofenac sodium–β-cyclodextrin inclusion complex

The inclusion complex of diclofenac sodium with β-cyclodextrin was prepared by the kneading method. β-Cyclodextrin, 1·135 g, was wetted with ethanol in an agate mortar and kneaded to form a paste. Then 0·318 g diclofenac sodium and ethanol were added. The sample was kneaded for approximately 60 min and dried to constant mass at

105°C. The final product was characterized in the solid state by X-ray, DSC and by ^{13}C NMR.

Instrumentation

Characterization of the diclofenac sodium–β-cyclodextrin complex in the solid state was performed by X-ray using an X-ray diffractometer TUR M62. X-ray analysis was performed using nickel-filtered CuK_α radiation ($\lambda = 1{\cdot}5418$ Å). The voltage and current were 30 kV and 30 mA, respectively. The inclusion complex obtained was identified by comparing its X-ray patterns with those of pure β-cyclodextrin, diclofenac sodium and a physical mixture of β-cyclodextrin and diclofenac sodium.

DSC analysis was performed for diclofenac sodium, β-cyclodextrin, the physical mixture of the two at the molar ratio of 1 : 1, and the product of kneading. Thermograms were obtained by use of a Shimadzu DSC-50 instrument using vented aluminium pans. The analysis was performed in a nitrogen atmosphere. All samples were run at a scanning rate of $10°C\,min^{-1}$, from 20 to 480°C (Figure 2A–D).

^{13}C NMR spectra for analysis were taken on a Varian Uniti 300 spectrometer using the following parameters: ^{13}C frequency 75·43 MHz, frequency range 6000 MHz, memory 65 kB, pulse width 11·3 μs, acquisition time 0·999 s, resolution 0·3 Hz. Spectroscopic measurements of the samples of $0{\cdot}3\,mol\,L^{-1}$ concentration were performed in DMSO-d_6. The chemical shifts were determined with respect to the signal of the solvent (DMSO-d_6 39·500 ppm) and expressed for TMS, which was the internal standard.

Determination of diclofenac sodium in the inclusion complex with β-cyclodextrin

Samples of the complex (20 mg) were placed in measuring flasks, and then distilled water was added up to the volume 25 mL. The contents were mechanically shaken for 30 min. The concentration of diclofenac sodium in the solution was determined spectrophotometrically (Agatonovic-Kustrin et al 1997; Pérez-Ruiz et al 1997) at $\lambda_{max} = 275$ nm by use of a Cary 118 Varian UV-vis spectrophotometer. The amount of diclofenac sodium in the inclusion complex with β-cyclodextrin was calculated as the difference between the mass of weighed portions of the complex studied and the mass of diclofenac sodium determined spectrophotometrically in the solution. The amount of diclofenac sodium contained in 1-g inclusion complex with β-cyclodextrin was 0·2189 g.

Thermal stability of pure diclofenac sodium and that present in the inclusion complex with β-cyclodextrin

Thermal stability of diclofenac sodium and its inclusion complex with β-cyclodextrin was studied at 60, 70 and 80°C in dry air. The vials containing samples of either 0·0250 g diclofenac sodium or 0·1142 g inclusion complex were placed in heated chambers placed in sand baths at the given temperatures.

At certain time intervals the contents of the vials were quantitatively transferred to 25-mL measuring flasks, which were made-up with water to the mark. After filtering, the concentration of diclofenac sodium was determined spectrophotometrically.

HPLC of the decomposition products of diclofenac sodium alone and in the inclusion complex with β-cyclodextrin

Analysis of the decomposition products of pure diclofenac sodium and diclofenac sodium in the inclusion complex with β-cyclodextrin, stored at 80°C, was performed by an HPLC method (Kubala et al 1993; Mason & Hobbs 1995). A high-performance liquid chromatograph (Shimadzu Co., Kyoto, Japan) was used with a stationary phase of LiChrosorb RP-18. The mobile phase was a mixture of acetonitrile–water (50 : 50, v/v), pH adjusted to 3·3 with acetic acid. The detector wavelength was set at 276 nm, the flow rate of the mobile phase was $1{\cdot}5\,mL\,min^{-1}$ and the column temperature was 20°C.

GC–MS analysis of the decomposition products of diclofenac sodium alone and in inclusion complex with β-cyclodextrin

Before the GC–MS studies, the samples were separated into fractions by extraction to solid state. Two millilitres aqueous solution of the inclusion complex ($11{\cdot}4\,g\,L^{-1}$) or diclofenac sodium ($2{\cdot}5\,g\,L^{-1}$) was placed onto the column packed with octadecylsilane phase C-18, previously conditioned with 5 mL methanol. The elution was performed with: 2 mL 1 : 1 methanol–water solution; 2 mL 4 : 1 methanol–water solution; 2 mL methanol.

The presence of the decomposition products in the eluate was searched for by a TLC method on plates coated with F254 silica gel (0·25-mm thick). The fractions collected were dried at room temperature in a nitrogen atmosphere to dry mass. The dry mass was dissolved in acetone and then 20 mL of this prepared solution was injected onto the column.

The parameters of the gas chromatograph (Hewlett-Packard 5890 series H, USA) were as follows. The capillary column was a DB-5 (30 m × 0·25 mm) Folson Ca (USA), the carrier gas was helium at a flow rate of 1 mL min^{-1}, and the detector was a Hewlett Packard 5971 A mass spectrometer (USA).

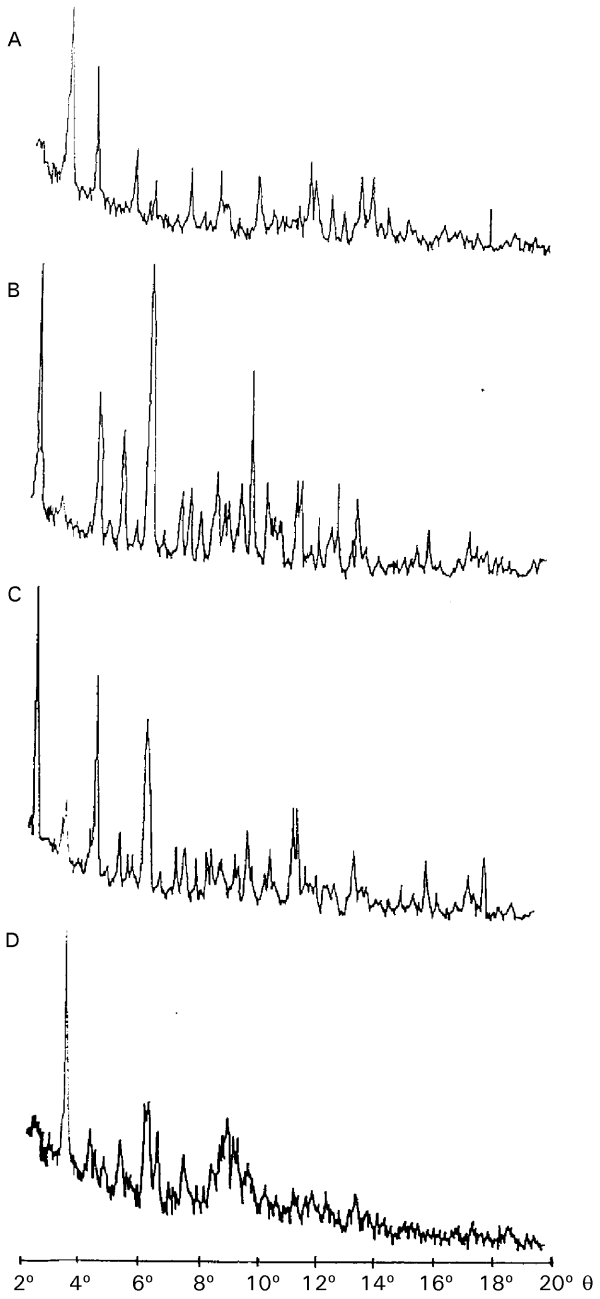

Figure 1. X-ray diffraction patterns. A, diclofenac sodium; B, β-cyclodextrin; C, physical mixture of diclofenac sodium and β-cyclodextrin; D, diclofenac sodium–β-cyclodextrin inclusion complex.

Results and Discussion

For the X-ray diffraction results the Debye reflections were measured in the θ angle range from 2 to 20°. The spectrum of β-cyclodextrin (Figure 1B) when compared with diclofenac sodium (Figure 1A) showed a higher number of reflections of higher intensity. The interplanar distances corresponding to the high intensity reflections were 19·56, 14·35, 9·96, 7·60 and 6·02 Å. The X-ray diffraction pattern of diclofenac sodium revealed fewer high intensity reflections and they corresponded to the following interplanar distances: 13·31, 10·43, 9·81 and 9·33 Å. The pattern of the physical mixture of diclofenac and β-cyclodextrin (Figure 1C) showed the peaks characteristic of both β-cyclodextrin (19·56, 9·96, 7·60 Å) and diclofenac sodium (13·31, 9·33 Å). The X-ray diffraction pattern of the kneading product (Figure 1D) was poor in reflections in the angle range of 15–20°, which testified to a reduced ordering of the crystal lattice. The fragment of the pattern corresponding to the other angle range revealed the peaks characteristic of the inclusion complex which corresponded to the interplanar distances of 12·05, 8·86, 4·80 Å. Their appearance indicated the formation of a new crystal

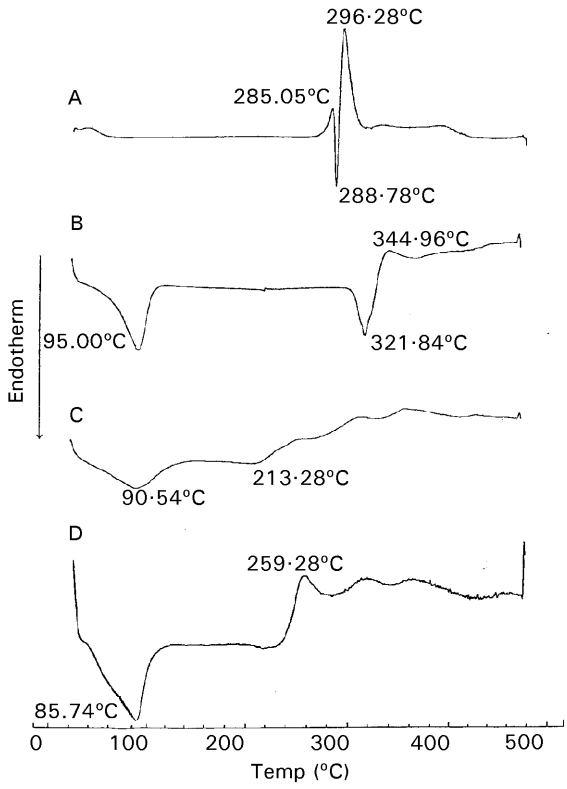

Figure 2. Differential scanning calorimetry curves of diclofenac sodium–β-cyclodextrin system. A, Diclofenac sodium; B, β-cyclodextrin; C, physical mixture of diclofenac sodium and β-cyclodextrin; D, diclofenac sodium–β-cyclodextrin inclusion complex.

lattice of the inclusion complex of diclofenac sodium and β-cyclodextrin.

The formation of such an inclusion complex was also confirmed by DSC studies. The thermogram of diclofenac sodium (Figure 2A) showed a single endothermic peak at 288·78°C, corresponding to the melting point of this compound. Between 283–285°C diclofenac sodium begins to melt and decompose, so the peak at 285°C pointed to the beginning of the decomposition process. The thermogram of β-cyclodextrin (Figure 2B) showed two endothermic peaks at 95°C and 321·84°C, the former corresponding to the water evaporation from β-cyclodextrin cavities, the latter corresponding to the decomposition of the β-cyclodextrin molecule.

In the thermogram of the inclusion complex of diclofenac sodium and β-cyclodextrin (Figure 2D), the endothermic peaks characteristic of β-cyclodextrin and diclofenac sodium disappeared, whereas the exothermic peak at 259·28°C, which did not occur in any other thermograms, appeared. The presence of this peak was evidence for the formation of the inclusion complex.

Another method used to confirm the identity of the product obtained by the kneading of β-cyclodextrin and diclofenac sodium was ^{13}C NMR spectroscopy (Figure 3). The spectrum of the kneading product revealed changes in the chemical shift δ of the carbon atoms relative to their positions in the spectrum of pure diclofenac sodium. The position of the carbonyl carbon was shifted from 175·733 (diclofenac sodium) to 175·686 ppm (inclusion complex), so $\Delta\delta = 0{\cdot}047$ ppm. Moreover, the position of the carbon atom of the $CH_2-COONa$ group was shifted from 44·339 ppm (diclofenac sodium) to 43·871 ppm (inclusion complex), so Δδ was 0·468 ppm. The changes suggested that the cavities in β-cyclodextrin were filled with the acetate groups of diclofenac sodium and that the inclusion complex was formed.

To determine the thermal stability, the samples were stored at 60, 70 and 80°C for 24 months and the effect of these temperatures was analysed. The results are presented as semilogarithmic plots of $\ln c = f(t)$, where c is parent compound concentration and t is time (Figure 4). The results proved that the decomposition of diclofenac sodium both alone and in inclusion complex with β-cyclodextrin occurred according to the simple equation of the first-order reaction.

The kinetic parameters of the decomposition reaction, given in Table 1, proved that the thermal stability of diclofenac sodium stored in each of the three temperatures was much higher when it was in the form of inclusion complex with β-cyclodextrin. This conclusion was supported by the thermodynamic parameters given in Table 2.

To determine the number of decomposition products, the two forms of diclofenac sodium stored at an elevated temperature (80°C) were subjected to

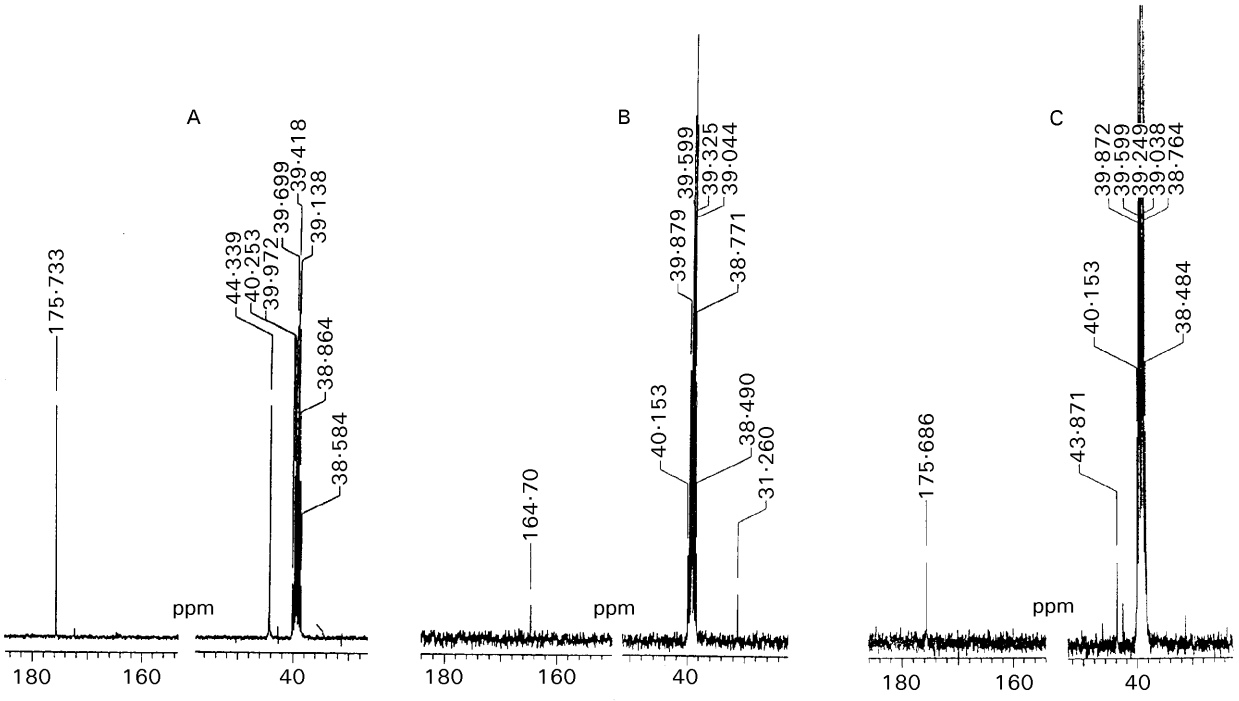

Figure 3. ^{13}C NMR spectra of diclofenac sodium (A), β-cyclodextrin (B), and diclofenac sodium–β-cyclodextrin (C) inclusion complex.

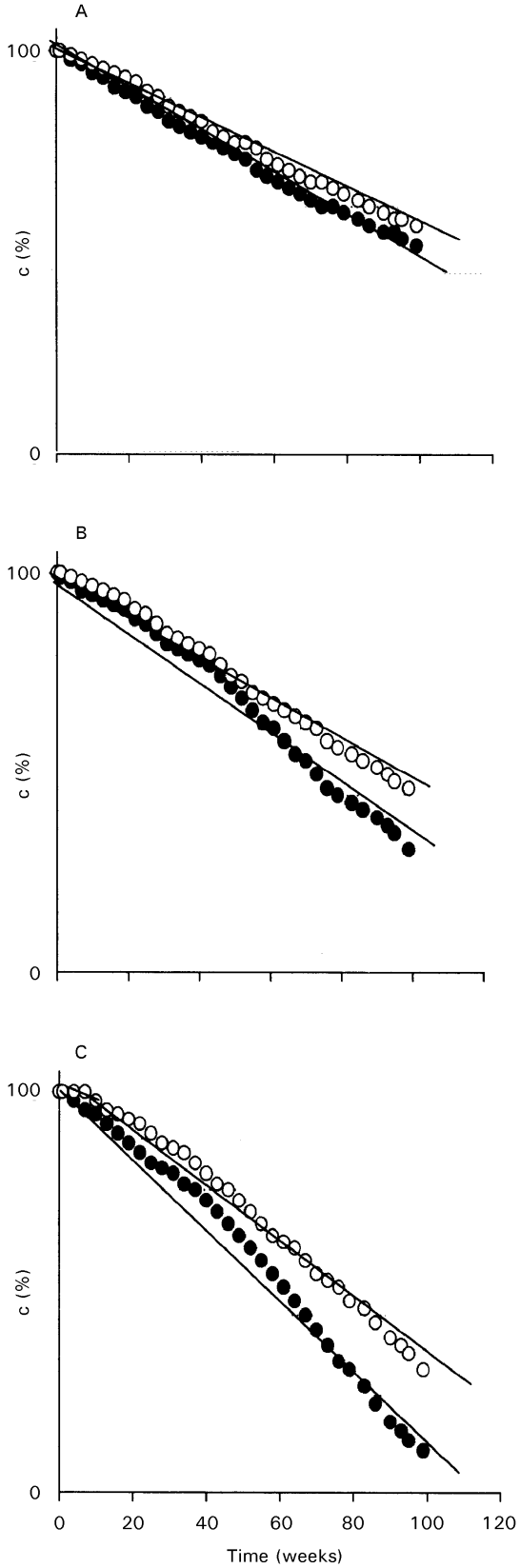

Figure 4. Thermal degradation of diclofenac sodium as a function of time at 60 (A), 70 (B) and 80°C (C). ● Diclofenac sodium, ○ inclusion complex.

Table 1. Kinetic parameters of diclofenac sodium alone and in inclusion complex with β-cyclodextrin in solid state.

		Kinetic parameters		
	Temperature K	10^9 k (s^{-1})	$t_{0.1}$ (d^{-1})	$t_{0.5}$ (d^{-1})
Diclofenac sodium	333	3·264 ± 0·07	441·0	2899·6
	343	4·701 ± 0·14	313·7	2062·5
	353	6·147 ± 0·21	213·4	1402·8
Inclusion complex	333	3·072 ± 0·06	511·6	3364·1
	343	3·821 ± 0·05	364·7	2397·9
	353	4·896 ± 0·14	236·8	1557·3

HPLC analysis. The chromatograms of the samples revealed the presence of five decomposition products ($t_R = 1·996$, 5·900, 8·510, 14·050, 26·307). The retention time of diclofenac sodium was 12·315 min and that of β-cyclodextrin was 13·367 min (Figure 5).

The same number of decomposition products was obtained for uncomplexed diclofenac and for that in the inclusion complex with β-cyclodextrin. This suggested that the mechanism of its decomposition was the same for the conditions studied. This meant that β-cyclodextrin did not interfere with the reaction of diclofenac sodium decomposition.

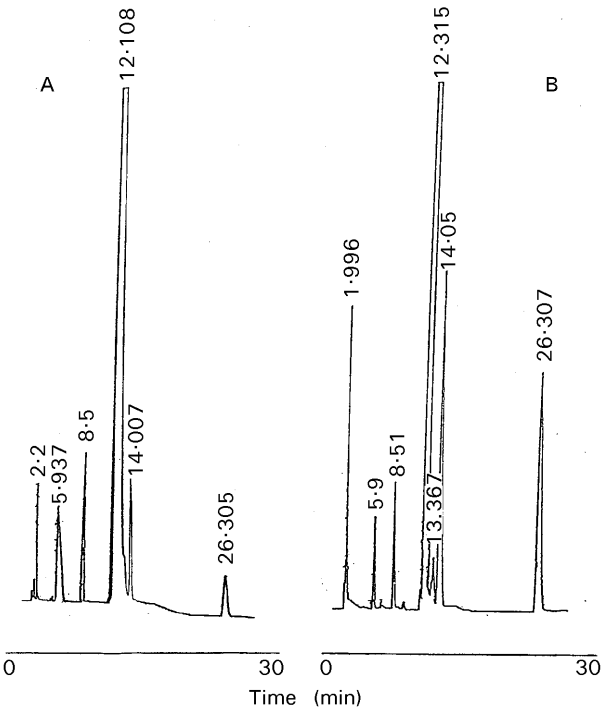

Figure 5. Chromatograms of samples stored at 80°C for 24 months obtained by HPLC. A. Diclofenac sodium uncomplexed and B. diclofenac sodium–β-cyclodextrin inclusion complex.

Table 2. Thermodynamic parameters for the degradation reaction of diclofenac sodium alone and in the inclusion complex with β-cyclodextrin in the solid state.

	Thermodynamic parameters					
	a	lnA	r	E_a (J mol^{-1})	ΔH (J mol^{-1})	ΔS (J mol^{-1}·K^{-1})
Diclofenac sodium	-3728 ± 1472	$-8{\cdot}33 \pm 4{\cdot}29$	$-0{\cdot}9980$	30996 ± 12235	28228 ± 12235	-315 ± 36
Inclusion complex	-2738 ± 848	$-11{\cdot}38 \pm 2{\cdot}48$	$-0{\cdot}9988$	22765 ± 7057	19997 ± 7057	-340 ± 21

a, slope; lnA, coefficient frequency; r, regression coefficient; E_a, activation energy; ΔH, enthalpy; ΔS, entropy. $n = 6$; $\alpha = 0{\cdot}05$.

To identify the products of decomposition, they were subjected to GC–MS analysis. The substances were separated into fractions by extraction to solid phase. The greatest number of compounds more hydrophobic than diclofenac sodium was found in the fraction extracted with 50% of methanol.

The chromatograms obtained as a result of gas chromatographic separation showed five peaks corresponding to the products of diclofenac sodium decomposition: $t_R = 15{\cdot}58$, 16·86, 17·21, 19·67 and 20·34 min. The mass spectra of the products were recorded and the products were identified on the basis of the mass of the molecular ion and the fragment ions. Four of the five identified compounds were described by Sun & Fabre (1994). Those authors presented a general scheme of diclofenac sodium decomposition. The compounds we found were 1-(2,6-dichlorophenyl)oxindole, 1-(2,6-dichlorophenyl)isatin, 2-[(2,6-dichloro-phenyl) amino]benzyl alcohol, and *N*-(2,6-dichlorophenyl) anthranililaldehyde, the same as those found by Sun & Fabre (1994). Our fifth compound was 4-chloro-10H-
9-acridinone, to the best of our knowledge not reported previously in the literature as a product of diclofenac sodium decomposition.

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