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THE IDENTIFICATION BY MS AND GC/MS OF PHOTO-DEGRADATION PRODUCTS OF INDOMETHACIN OINTMENT

VASILE MICLĂUŞ[1], ILDIKÓ BROŞ[2], ZAHARIA MOLDOVAN[2], CLAUDIA CIMPOIU[1], EMANOIL SURDUCAN[2], NICOLAE PALIBRODA[2]

[1]Babeş-Bolyai University, Faculty of Chemistry
[2]National Institute R&D for Isotopic and Molecular Technologies

ABSTRACT. This paper reports the photo degradation of indomethacin from a pharmaceutical cream formulation. For the determination of the degradation compounds a MS analysis was performed by direct introduction of the sample in high vacuum and a GC separation of the components. Mass spectra of electronic impact (EI) in GC-MS were also registered. Six compounds, which held the indomethacine moieties, were identified.

INTRODUCTION

Indomethacin ([1-(4-chlorobenzoyl)-5-methoxy-2-methyl-indol-3-yl] acetic acid) was introduced to the market in 1963, and since then it has been widely used in musculoskeletal and joint disorders such as rheumatoid arthritis, ankylosing spondylitis, osteoarthrithis and gout. Its potency as an anti-inflammatory, analgesic, antipyretic agent is counterbalanced by accompanying adverse effects [1-4].In the course of the 40-year history of indomethacinseveral papers have been published on its stability (including photostability) and the identification of its degradation products. Some of them are mentioned in the paper. With the exception of the Krzek-Starek paper(which takes into account the kinetics of the degradation of indomethacin in basic medium [4], all deal with photostability/photochemical degradation products of indomethacin [5-6]. So far little has been published on impurities and photo degradation products of the formulations.

This paper reports the photo degradation of an indomethacin ointment and determination of the products by a) mass spectrometry (MS) with direct insert of the sample in high vacuum and b) gas chromatography coupled with electronic impact mass spectrometry GC-MS(EI).

EXPERIMENTAL

1). Degradation of an indomethacin ointment

The degradation tests were performed on an indomethacin cream formulation. The ointment was subjected to irradiation by daylight for 60 days at room temperature. The degradation occurred simultaneously with the colour change.

The indomethacin and a part of the by-products contained in the photo-degradated ointment were transformed in soluble sodium salts with a diluted NaOH solution. For 35 g ointment were added 0,1565 g NaOH and 50 ml distilled water. For the obtained yellow emulsion, the pH was verified to be 7,5 - 9. This emulsion was washed with CH_3OH and filtered in vacuum. The filtrate, with a yellow colour was treated with diluted H_2SO_4 to a weak acidic medium (pH=6-6,5), and then

extracted with diethyl ether. After ether evaporation, a mixture of solid brownish crystals were obtained. GC/MS investigation revealed that in addition to indomethacin the extract contained fatty acid esters originating from the base cream .

2). Separation and determination of the degradation compounds

A separation of these compounds by thin layer chromatography (TLC) on Kieselgel 60 F_{254} plates was performed.The solution was obtained by solving 1 mg extract in 1 ml methanol. The solution was applied with a micropipette as a spot on the plate. The plate was developed in an ascendant way using the mixtures of chloroform: methanol (3:1,V/V); chloroform: diethyl ether (1:1,V/V), n-butanol: water: glacial acetic acid (4:1:1, V/V/V) as mobile phase. After elution, the plates were dried in air and the spots were detected in UV light (λ=254 nm).

For the spectrometric mass analysis, a mass spectrometer MAT 311 was used, with direct introduction of the sample in high vacuum. The photodegradated indomethacin extract, in the solid phase, was introduced in a heated crucible into the high vacuum, with a programme of temperature ranging from 20 to 250 ^{0}C and a simultaneous registration of the mass spectra in repetitive scanning.

For chromatographic separation of photo degradation products a gas chromatograph Hewlett-Packard type 5840 A equipped with a capillary column of 30 m length of a DB5 stationary phase was used. The sample was injected as a methanol solution. The mass spectra were registered with a quadrupolar mass spectrometer HP 5985 with electronic ionisation (EI) for the mass range 35 - 400 dalton.

RESULTS AND DISCUSSION

The investigations by thin layer chromatography (TLC) could not offer conclusive identification of the separated spots. The TLC separation of degradation products emphasized the presence of some partially separated substances.

Figure 1 presents the fragmentograme of the degradation products of the extract, obtained by the direct introduction method in MS.

The sample components sublimed between 60–170 ^{o}C, the maximum of the total ions curve being recorded at 110 ^{o}C. Figure 1 put into evidence the sublimation of four components in vacuum.

The first component **a**, the most volatile, characterized by the ions with the mass m/z = 139 and 156 (M^+), represents p-chlorobenzoic acid (**A**). The mass spectrum of this compound is analogous to the mass spectrum known from the literature [6]. This compound derives from indomethacin either from the hydrolysis or from the fragmentation (**A**, Scheme 1).

The ions with the mass m/z = 174 and 219 describe the second degradation product (**b**), with the mass spectrum presented in Figure 2.

For this compound structure **B** from Scheme 1 was attributed. The main fragment, the ion m/z = 174 derives from the cleavage of the carboxyl function (M^+ - 45).

From the quantitative point of view, the compounds A and B represent the principal products of fragmentation.

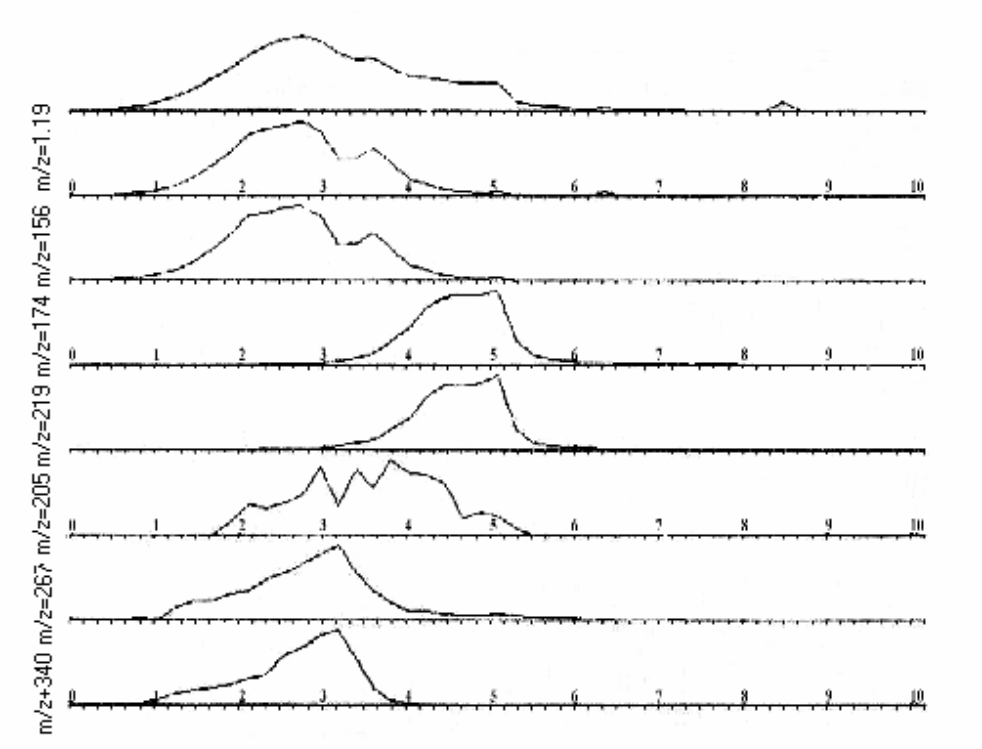
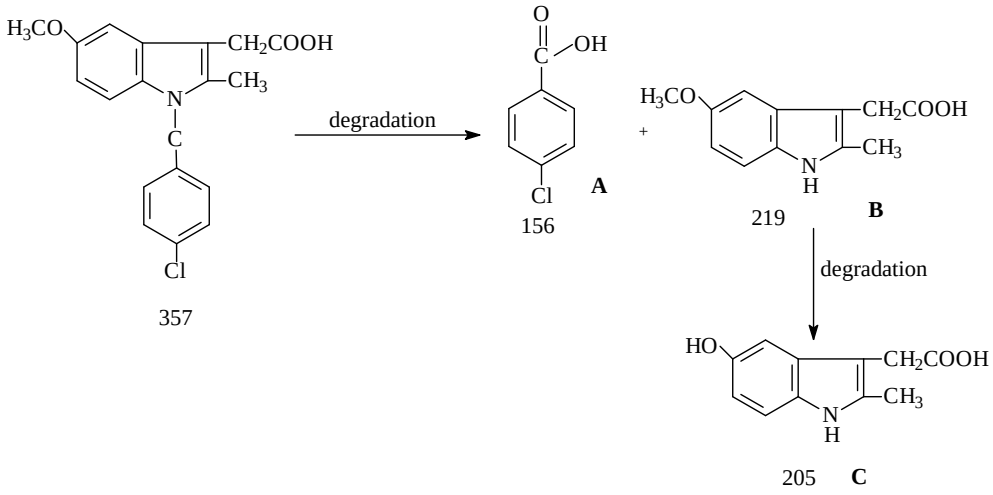


Fig. 1. The fragmentograme of the degradation products of indomethacin extract in direct MS



Scheme 1

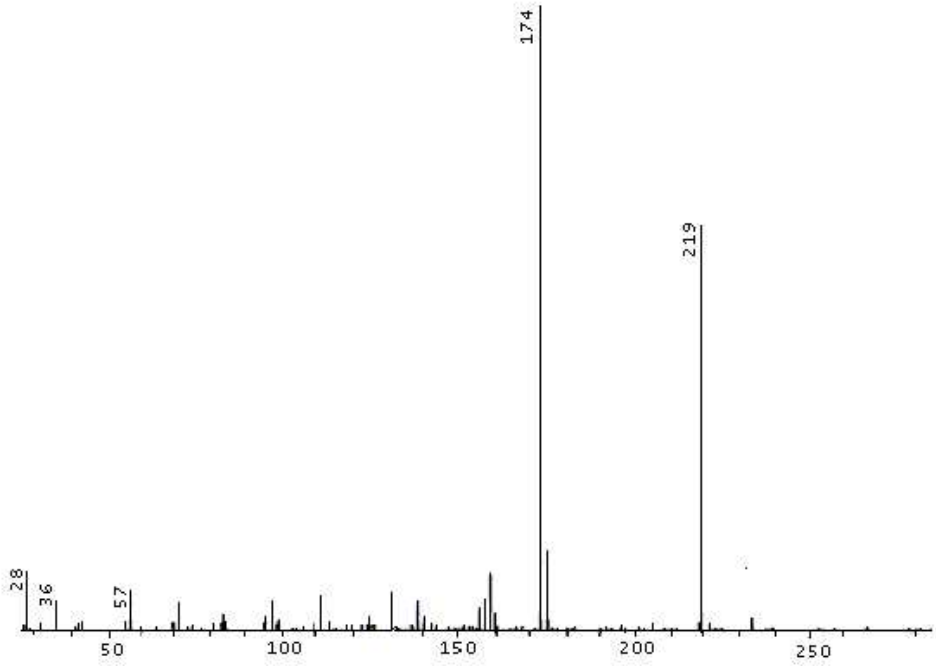


Figure 2. The Mass Spectra of compound **B**.

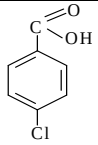
A third compound **c**, present in small amount, is represented in Figure 1 of the fragmentogram at the mass m/z = 205, with a maximum of sublimation in vacuum at 120 ^{o}C. A clean mass spectrum of this compound could not be obtained, due to the small intensity of the ions. A credible structure of the compound **c** could be the formula **C** from scheme 1 with M^{+}=205, obtained after demetilation of **B**.

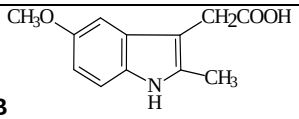
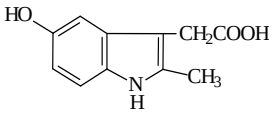
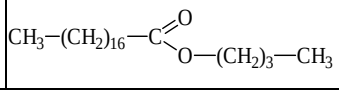
The last two fragmentogrames at the masses m/z = 267 and 340 represent the fragment ion M^{+}– 73 and the molecular ion M^{+} = 340 of butyl stearate which is found in creme composition.

The degradation products are summarized in Table 1.

Tabel 1

Some data of the determined structures (**a-d**) from direct MS

Subst.	Abundance %	Molecular mass	Molecular Formula	Structural formula
a	55.755	156	$C_7H_5O_2Cl$	 **A**

Subst.	Abundance %	Molecular mass	Molecular Formula	Structural formula
b	40.628	219	$C_{12}H_{13}O_3N$	 **B**
c	0.095	205	$C_{11}H_{11}O_3N$	 **C**
d	3.522	340	$C_{22}H_{44}O_2$	

The gas chromatogram of the methanol solution of the degradation products of indomethacin, obtained with a flame ionisation detector, is presented in Figure 3.

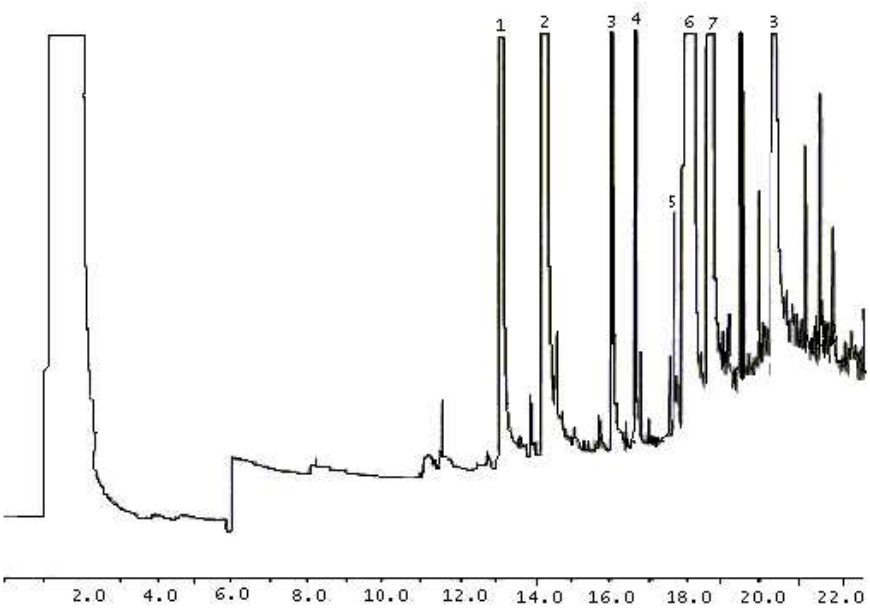


Figure 3. The gas chromatogram (GC) of the methanol solution of indomethacin degradation products

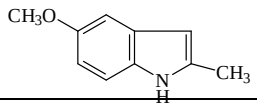
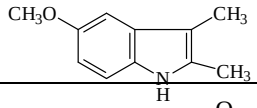
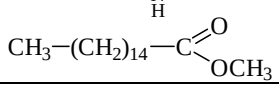
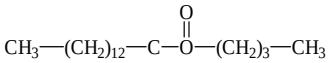
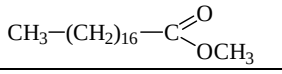
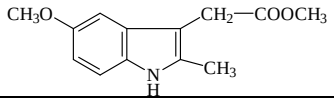
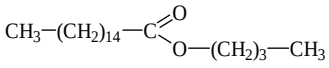
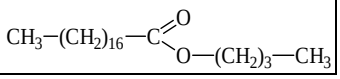
The mass spectra allowed the identification of five esters (3,4,5,7,8,) originating from the ointment, with the structures shown in Table 2.

The peaks numbered **1**, **2** and **6** represent the indomethacin photo degradation compounds, with structures **D, E** and **F** respectively.

The compound **F** with M^+=233 is equivalent to the structure **B** (Scheme 1), but is methylated at the carboxyl function. Structure **B** could be methylated in the gas phase, either from the methanol solution or/and from the methyl fragments derived from temperature degradation of the substances along the column.

Table 2

The products identified through GC-MS(EI) from the sample

Nr. peak	Retention time	Molecular mass	Molecular Formula	Structural formula
1.	13'	161	$C_{11}H_{11}NO$	**D** 
2.	14'15"	175	$C_{11}H_{13}NO$	**E** 
3.	16'10"	270	$C_{17}H_{34}O_2$	
4.	16'50"	284	$C_{18}H_{36}O_2$	
5.	17'45"	298	$C_{19}H_{38}O_2$	
6.	18'50"	233	$C_{13}H_{15}NO_3$	**F** 
7.	19'30"	312	$C_{20}H_{40}O_2$	
8.	20'25"	340	$C_{22}H_{44}O_2$	

CONCLUSIONS

For the determination of the photo degradation compounds of an indomethacin extract from the ointment, a MS analysis by direct introduction of the sample in high vacuum and a GC separation of the components was performed. Mass spectra in GC-MS (EI) were also registered.

These two methods are unable to reveal the presence of undegradated indomethacin in the extract. Out of six identified structures, five (**B, C, D, E, F**) retained the indol moieties.

Degradation products **A, B** and **E** are known degradants of indomethacin (products of hydrolytic splitting and decarboxylation). The structure **B** was the same as detected in the photooxidation of this drug, resulting from decarboxylation [7]. The revealed fragmentations for the other newly detected compounds (**C, D, E, F**), are partially analogues to the conversions of indomethacin in metabolic processes known in the literature [8].

There is no evidence that **C**, which appears as a minor component (0.1%>) in the course of the direct MS investigation and does not appear in the GC/MS scan, is a photodegradation product. **D** is an interesting, unusual structure but there is no clear evidence that the structure is really what is presented in Table 2: it could also be the isomeric 5-hydroxy-2,3-dimethyl or 5-methoxy-3-methyl derivative. In the same time we assume that **F** could be a GC/MS artefact.

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