ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 499–509

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Identification of degradation products of Ibuprofen arising from oxidative and thermal treatments

Gabriele Caviglioli [a,*], Posocco Valeria [a], Parodi Brunella [a], Cafaggi Sergio [a], Alzati Attilia [b], Bignardi Gaetano [a]

[a] *Dipartimento di Chimica e Tecnologie Farmaceutiche e Alimentari, Università degli Studi di Genova, Via Brigata Salerno (ponte), 16147 Genova, Italy*

[b] *Pharmatec International S.r.l., via Molise, 16, 20098 S. Giuliano Milanese, Italy*

Received 19 February 2001; received in revised form 20 April 2002; accepted 26 April 2002

Abstract

Ibuprofen is a widely utilised analgesic anti-inflammatory drug. It is sensitive to oxidation and photodegradation. In this work, the oxidative and thermal degradations were investigated. The treatments adopted allowed the detection of 13 degradation products, seven of which have never been reported: hydratropic acid, 4-ethylbenzaldehyde, 4-(1-carboxyethyl)benzoic acid, 1-(4-isobutylphenyl)-1-ethanol, 2-[4-(1-hydroxy-2-methylpropyl)phenyl]propanoic acid, 1-isobutyl-4-vinylbenzene, 4-isobutylphenol. For 1-(4-isobutylphenyl)-1-ethanol, the in vitro toxic effects have already been described in the literature. To detect all degradation products, two RP-HPLC methods and a GC-MS procedure were developed or modified from the official monographs. The identification was conducted by evaluating chromatographic and spectral data and the structural attributions were confirmed by simple and univocal synthesis. Moreover, the actual presence of these molecules in marketed medicinal products was investigated.

Keywords: Ibuprofen; Degradation products; Oxidation; HPLC; GC-MS; Methyl derivatives

1. Introduction

Ibuprofen (IBU) is a nonsteroidal anti-inflammatory drug belonging to the group of propionic acid derivatives. It is widely used for painful and inflammatory conditions and is available for over-the-counter (OTC) sale. The usual dose, from 200 to 1200 mg daily, can be increased for prescription use to 3200 mg in divided portions [1,2]. While prescription use in the US appears to be stabilising, non-prescription sales of IBU have more than tripled since it was approved as an OTC drug in 1984 [3]. The only available data on IBU consumption refer to the 1990 US market, when it amounted to 3000 t [4]. In 1998, a generic product containing IBU ranked twenty-fifth among the 200 most often prescribed proprietary name medicinal products in the US [5].

* Corresponding author. Tel.: +39-010-353-2635; fax: +39-010-353-2684

E-mail address: caviglioli@dictfa.unige.it (G. Caviglioli).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00400-4

In view of the widespread use or abuse of IBU as an OTC medication and the extensive consumption for chronic diseases such as arthritis, the presence of every possible IBU degradation product must be monitored for its long term toxicity. From the chemical standpoint, IBU is sensitive to oxidative [6] and photolytic [7,8] degradations which, following a radical mechanism, yield a number of products [9,10]. It is important to bear in mind that at least two degradation products, 4-isobutylacetophenone and 1-[4-isobutylphenyl]-1-ethanol, have shown, respectively, in vivo and in vitro toxic effects [7,11].

In this work, the existence of new products deriving from IBU decomposition was studied. The degradation was obtained submitting IBU, in solid state or in solution, to thermal treatments or to oxidising agents. Moreover, the effect of some pharmaceutical excipients on IBU stability was evaluated. To obviate the well-known influence of light on IBU stability [7], all degradations were carried out in the dark. The bulk was likewise stored in the dark.

Trace levels of these new degradation compounds were detected in the forced degradations of some marketed proprietary medicines. The degradation products were detected by two RP-HPLC methods and a GC-MS procedure. The main HPLC method derived from the official US and European monographs [10,12]. The identification was performed by chromatographic and spectral methods and the structural attribution confirmed by unambiguous synthesis.

2. Experimental

2.1. Chemical and materials

Ibuprofen, 2 - (4 - isobutyrylphenyl)propanoic acid, 2-(4-formylphenyl)propanoic acid, 4-acetylbenzoic acid, 4-isobutylbenzoic acid, 1-(4-isobutylphenyl)-1-ethanone and 1-(4-acetylphenyl)-2-methyl-1-propanone were supplied by Pharmatec International S.r.l. (Milan). Hydratropic acid, 4-ethylbenzaldehyde, isobutylbenzene, valerophenone, *N*-methyl-*N*^{1}-nitro-*N*-nitrosoguanidine, sodium borohydride, retention index mixture for GC, boron trifluoride-methanol 14 and 50% solution, and MNNG diazomethane generation apparatus were supplied by Sigma Aldrich S.r.l. (Milan). All other chemicals and solvents used were of analytical or HPLC grade.

2.2. Instruments

The HPLC system was an HP 1090 series II equipped with a DAD. The GC was an HP 5890 series II coupled to an HP 5971 A mass detector (Hewlett Packard, USA).

2.3. HPLC

2.3.1. HPLC method A

In order to identify IBU and test its purity, the standard and sample solutions were prepared in acetonitrile, weighing an accurate quantity to obtain solutions having a known concentration of 5.0 mg ml^{-1}.

The degradation products were also identified by relative retention time (RRT) based on valerophenone retention time. For this purpose, an acetonitrile solution of valerophenone having a concentration of $\approx$0.3 mg ml^{-1} was used to prepare degradation product standard solutions having a known concentration of $\approx$0.05 mg ml^{-1}.

Solution volumes of 2.5 μl were injected under ambient conditions onto a Superspher 100 (Merck) RP-18 (4 μm, 2 × 125 mm) cartridge column. The flow rate was 0.250 ml min^{-1} and the run time was 70 min. Eluant A was water, adjusted to pH = 2.5 with orthophosphoric acid, eluant B was acetonitrile, both filtered and degassed. After the injection, B was maintained at 26% for 9 min, afterwards it was changed to 30% and then kept at this composition for 32 min. Then the proportion of B was increased linearly to 88% in 29 min. The minimum column reequilibration time was set at 10 min. The analytical DAD wavelengths were set at 214, 240 and 257 nm with a bandwidth of 4 nm, and the reference wavelength was set at 450 nm with a bandwidth of 80 nm.

2.3.2. HPLC method B

The samples were prepared by weighing an accurate amount of IBU in order to obtain solutions having a known concentration of 1 mg ml^{-1} in mobile phase.

To identify the degradation products, standard solutions with a known concentration of ≈ 0.01 mg ml^{-1} of each product was prepared in the same solvent. The RRT calculation was based on 2-(4-formylphenyl)propanoic acid retention time.

Solution volumes of 25 μl were injected at ambient temperature onto a Water μBondpack Phenyl (10 μm, 3.9×300 mm) column. The flow rate was 1.250 ml min^{-1} and the run time was 40 min. The mobile phase consisted of methanol–ammonium acetate (0.15 M) (60:940 v/v) adjusted to apparent pH 4 with acetic acid. The mixture was filtered and degassed. The analytical DAD wavelengths were set at 257, 240 nm with a bandwidth of 4 nm and the reference wavelength was at 450 with a bandwidth of 80 nm.

2.4. GC-MS

2.4.1. GC-MS method

To evaluate IBU purity, 0.5 mg of sample were submitted to derivatization with 1 ml of 2 N methanolic hydrogen chloride heating at 60 °C for 20 min. Methyl derivatives were extracted for 10 min by 1 ml of hexane and organic solution directly injected into GC.

Degradation products were identified by preparing hexane solutions of standards or their methyl derivatives, with a concentration of $\approx$ 0.01 mg ml^{-1}. The GC, equipped with EI mass detector, was tuned with perfluorotributylamine. The instrument contained a 25 m $\times$ 0.2 mm (i.d.) fused-silica column supporting a cross-linked methyl siloxane bonded phase (thickness 0.3 μm) (HP1, Hewlett Packard). Helium was employed as carrier gas at the constant pressure of 16.4 psi measured at the on-column injector port. The injector temperature was set to follow the oven temperature program. 1.0 μl of hexanic solution, containing methyl derivatives or volatile compounds, was manually injected with solvent delay set to 2.3 min. After an initial hold time of 0.2 min at 40 °C, the oven temperature was programmed to 250 °C at the rate of 15 °C min^{-1} and held isothermally for 5 min. The mass range was scanned from 40 to 300 a.m.u. every 2.6 s and the ions with relative abundance higher than 500 were stored. Retention index values were calculated applying the Van den Dool and Kratz equation [13] to programmed-temperature retention times.

2.4.2. Methylation methods

2.4.2.1. Hydrogen chloride in methanol. 1.0 ml of 1 N or 2 N hydrogen chloride in methanol was added to the sample. The mixture was vortexed for 1 min and heated at 60 °C for 20 min or allowed to stand at room temperature for 1 h. After saturation with sodium chloride, methyl esters were extracted for 10 min by 1.0 ml of hexane.

2.4.2.2. Boron trifluoride in methanol. A total of 1.0 ml of 14 or 50% of boron trifluoride in methanol was added to the sample. The mixture was vortexed for 5 min and heated at 60 °C for 30 min or allowed to stand at room temperature for 1 h. Five drops of water were then added and methyl esters were extracted for 10 min by 1.0 ml of hexane.

2.4.2.3. Diazomethane. The sample was treated with 250 μl of diazomethane in diethyl ether ($\approx$ 0.2 mmol ml^{-1}) for 12 min at ambient temperature. The mixture was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 1.0 ml of hexane vortexing for 1 min.

2.5. Trace amount detection of the degradation products in some proprietary name medicines containing Ibuprofen

Three units of each dosage form, deprived of coating if present, were finely powdered. An accurately weighed portion of the powder, equivalent to $\approx$250 mg of IBU, was extracted for 3 h with 50 ml of acetonitrile by stirring and periodic sonication. After centrifugation, the supernatant was filtered through a 0.45 μm cellulose acetate membrane and the solution was evaporated to dryness under reduced pressure at 40 °C. For

analysis by HPLC method A, the residue was dissolved in 0.5 ml of acetonitrile vortexing for 1 min. For the GC-MS method, the residue was submitted to derivatization by 2 N hydrogen chloride in methanol at 60 °C.

2.6. Degradation product synthesis

2.6.1. Synthesis of 4-(1-carboxyethyl)benzoic acid (code 7)

8 mg of 2-(4-formylphenyl)propanoic acid (code 5) were dissolved in 2 ml of 30% aqueous sodium hydroxide solution. The solution was heated at 40 °C for 12 h under stirring. Some drops of hydrogen chloride were then added to obtain acid pH and the mixture was extracted twice with 2 ml of ethyl acetate for 20 min. The organic solution was evaporated to dryness by nitrogen stream. The dismutation products contained in the residue were isolated by a semi-preparative TLC [6]. The coproduced alcohol, (2-[4-(hydroxymethyl)phenyl]propanoic acid), was also prepared by dissolving 3 mg of compound 5 and 12 mg of $NaBH_4$ in 1.0 ml of a mixture water and methanol (9:1). The solution was allowed to stand at room temperature for 1 h, then 0.5 ml of 10% hydrogen chloride were added and the compound was extracted with dichloromethane. The organic phase was evaporated to dryness by a nitrogen stream.

2.6.2. Synthesis of 1-(4-isobutylphenyl)-1-ethanol (code 2)

$NaBH_4$ (12 mg) was added to 3 mg of 1-(4-isobutylphenil)-1-ethanone (code 4) dissolved in 1 ml of ethanol. After dissolving the reduction agent by swirling, the solution was allowed to stand at room temperature for 1 h. 0.5 ml of 10% aqueous hydrogen chloride solution were then added and the mixture was extracted with hexane. The upper phase was evaporated to dryness by a nitrogen stream.

2.6.3. Synthesis of 2-[4-(1-hydroxy-2-methylpropyl)phenyl]propanoic acid (code 1)

A total of 12 mg of $NaBH_4$ and 1.5 mg of 1-(4-isobutyrylphenyl)propionic acid (code 3) were dissolved in 1 ml of H_2O–MeOH (50%) and the solution was allowed to stand at room temperature for 1 h. A total of 0.5 ml of 10% aqueous hydrogen chloride solution were then added and the mixture

Table 1
Degradative treatments to which Ibuprofen was submitted in solution and in solid state

Treatment[a]		T (°C)[b]	Time	Solvent	Excipient
A	$KMnO_4$ 0.05 N	RT	6 h	NaOH 0.5 M	
B	$KMnO_4$ 0.05 N	RT	9 days	NaOH 0.5 M	
C	$KMnO_4$ 0.65 N	RT	30 days	$NaHCO_3$ 0.5 M	
D	H_2O_2 16%	30°	3 h	Aceton	
E	H_2O_2 16%	30°	6 h	Methanol	
F	H_2O_2 33%	RT	22 h		
G	H_2O_2 16%	RT	3 days	NaOH 0.5 M	
H	$K_2Cr_2O_7$ 0.1 N	RT	14 days	HCl 0.5 M	
I	Heating (under reflux)	100°	4 h		
L	Heating (under reflux)	100°	7 h		
M	Heating (under reflux)	180°	16 h		
N	Heating (oven)	50°	7 days		
O	Heating (oven)	50°	8 months		
P	Heating (oven)	50°	8 months		PVP
Q	Heating (oven)	50°	8 months		PVP-Talc
R	Heating (oven)	50°	8 months		PVP-Explotab[c]
S	Heating (oven)	50°	8 days		PVP

[a] Treatments from A to H were conducted in aqueous medium, from I to S in the solid state.
[b] RT, room temperature.
[c] Sodium starch glycolate.

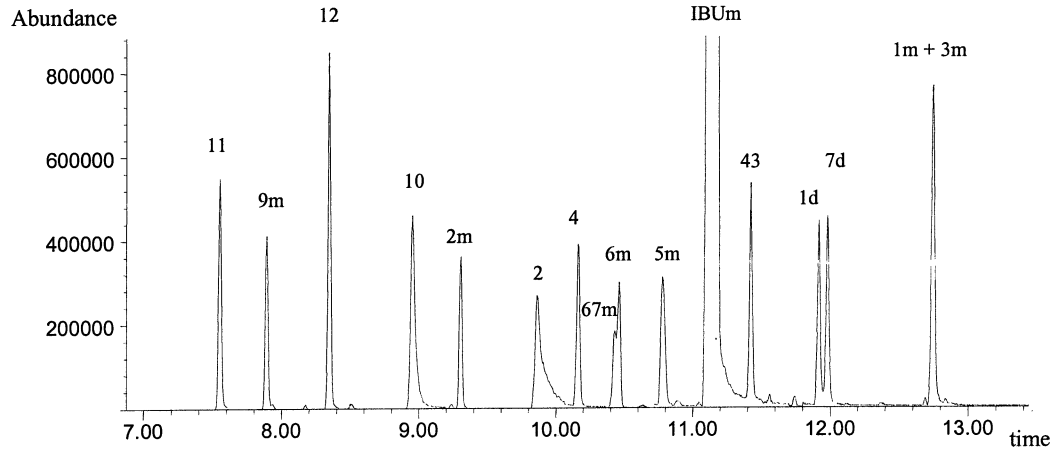

Fig. 1. Representative GC-MS chromatogram of Ibuprofen and its degradation products (as coded in Table 2). m, monomethyl derivative; d, dimethyl derivative.

was extracted with ethylacetate. The upper phase was evaporated to dryness by a nitrogen stream.

2.6.4. Synthesis of 1-isobutyl-4-vinylbenzene (code 12)

A total of 8 μl of 1-(4-isobutylphenyl)-1-ethanol (code 2) and 1 ml of 85% orthophosphoric acid were placed in a small round-bottomed flask and swirled to mix thoroughly. The reaction mixture was heated with a Bunsen burner and the alkene was collected over a 30 min period on the water cooled bulb of a Kugelrohr distillation apparatus. The obtained product, 1-isobutyl-4-vinylbenzene, was recovered by hexane and the solution evaporated to dryness by a nitrogen stream.

2.6.5. Synthesis of 4-isobutylphenol (code 10)

Isobutylbenzene (10 g) and 10 g of concentrated sulphuric acid were placed in a flask equipped with

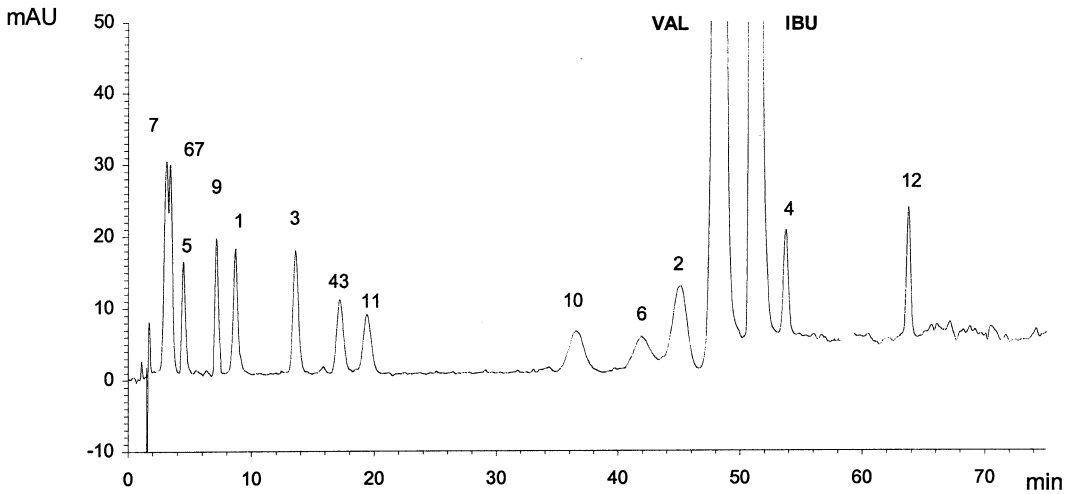

Fig. 2. Representative HPLC method A chromatogram of Ibuprofen (IBU) and its degradation products (as coded in Table 2) acquired at detection wavelength of 214 nm. VAL, valerophenone used as internal standard.

Table 2
Iupac names, main liquid chromatographic and spectral data of Ibuprofen degradation products

Code	IUPAC name	MW[a]	RRT[b] HPLC A	UV[c] λ_{max} A (nm)	RRT[d] HPLC B	UV[e] λ_{max} B (nm)
1	2-[4-(1-Hydroxy-2-methylpropyl)phenyl]propanoic acid	222	0.18	219		
2	1-(4-Isobutylphenyl)-1-ethanol	178	0.94	219		
3	2-(4-Isobutyrylphenyl)propanoic acid	220	0.28	251		
4	1-(4-Isobutylphenyl)-1-ethanone (or 4-isobutylacetophenone)	176	1.11	255		
5	2-(4-Formylphenyl)propanoic acid	178	0.09	257	1	261
6	4-Isobutylbenzoic acid	178	0.87	243		
7	4-(1-Carboxyethyl)benzoic acid	194	0.06	239	0.62	235
9	Hydratropic acid	150	0.15	205		
10	4-Isobutylphenol	150	0.76	223		
11	4-Ethylbenzaldehyde	134	0.4	261		
12	1-Isobutyl-4-vinylbenzene	160	1.32	253		
43	1-(4-Acetylphenyl)-2-methyl-1-propanone	176	0.36	259	1.3	247
67	4-Acetylbenzoic acid	164	0.07	251	0.53	253
IBU	2-(4-Isobutylphenyl) propanoic acid	206	1.06	221		
VAL	1-Fenil-1-pentanone (or Valerophenone)	162	1	243		

[a] MW, molecular weight.
[b] HPLC A relative retention time based on Valerophenone reference retention time.
[c] HPLC A maximum absorbance wavelength.
[d] HPLC B relative retention time based on 2-(4-formylphenyl)propanoic acid reference retention time.
[e] HPLC B maximum absorbance wavelength.

a reflux condenser and heated at 110–120 °C in an oil bath.

After 1 h, the reaction mixture was cooled to room temperature and 40 ml of cold water were poured into the flask. The filtered acidic solution was partially neutralised with sodium hydrogen carbonate, heated to boiling and filtered through a warmed Buchner funnel.

The filtered was cooled in ice and sodium isobutylbenzensulphonate crystals were separated by filtration and washed with a saturated sodium chloride solution to remove the small amounts of ortho and meta isomers. Potassium hydroxide pellets (10 g), together with 0.5 ml of water, were placed in a nickel crucible and heated in a muffle. At 250 °C sodium isobutylbenzensulphonate crystals were added to the melted mass and heated to 310 °C. The mobile, brown oil of the potassium salt of 4-isobutylphenol was taken from the pasty mass of alkali and ladled out on crushed ice. 4-Isobutylphenol was precipitated by adding concentrated hydrochloric acid, cooled in ice, filtered and dried.

2.7. Degradation procedures

The forced degradations were performed protecting samples from the light.

For degradations in solution, ≈20.0 mg of IBU were dissolved in 10 ml of each reagent and the tests were carried out as described in Table 1. The degradation products, after acidification with 5 N hydrochloric acid, were extracted with ethylacetate and organic solutions dried in rotavapor under vacuum at 40 °C. Thermal degradation in the solid state was evaluated on oven-stored IBU samples (≈50.0 mg) as described in Table 1. To allow the recovery of the more volatile degradation products, IBU powder (≈100.0 mg) was heated under reflux as described in Table 1. The influence of some excipients on IBU degradation

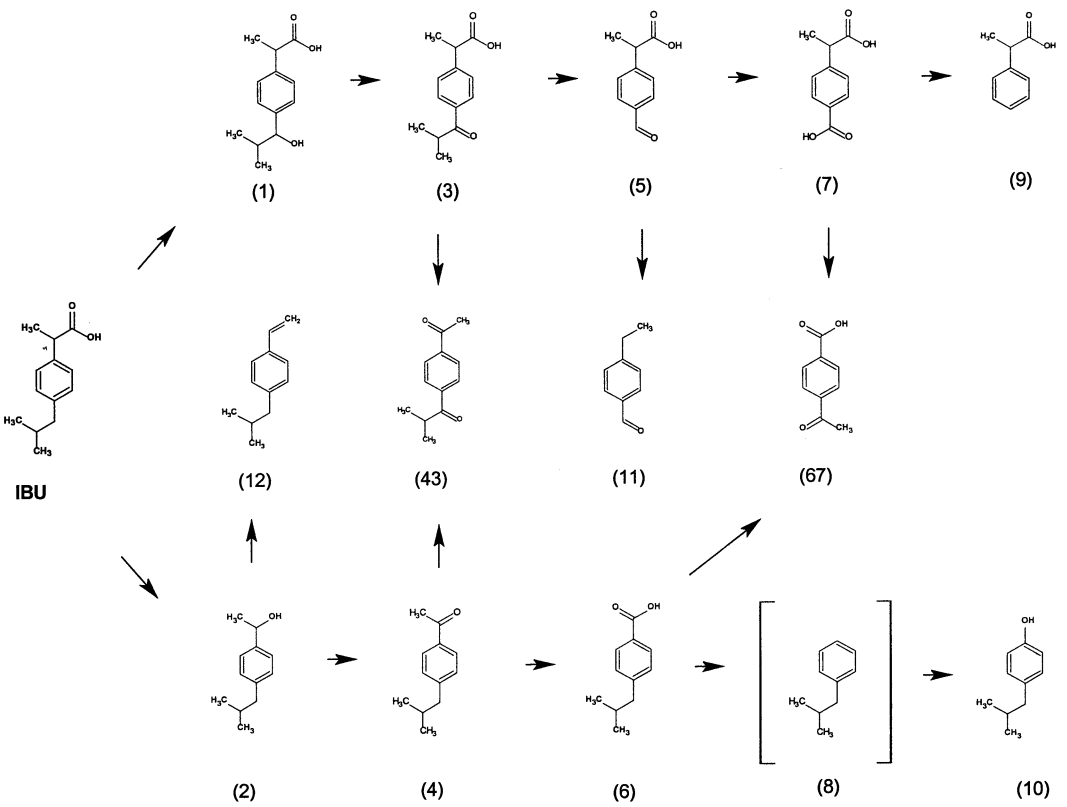

Fig. 3. Possible degradation scheme arranging new (1, 2, 7, 9, 10, 11, 12), known (3, 4, 5, 6, 43, 67) and hypothetical (8) Ibuprofen degradation products (as coded in Table 2).

was evaluated on mixtures containing the same amount (50 mg) of each component.

3. Results and discussion

Ibuprofen was submitted in solution and in the solid state to the different degradation conditions as listed in Table 1. The degraded samples were analysed by GC-MS (Fig. 1) and HPLC (Fig. 2: only method A) methods. The chromatograms showed various peaks in addition to that corresponding to IBU. The peaks 3, 4, 5, 6, 67 (Table 2, Fig. 3) were identified as known degradation products (KDP) [6,9] by comparison with HPLC relative retention time, GC retention index, mass and UV spectra with the corresponding reference standards (Tables 2 and 3). The peaks 1, 2, 7, 9, 10, 11, 12 were unidentified degradation products (UDP) or substances not yet correlated to the treatments described in Table 1. To demonstrate the real presence of UDP trace amounts in dosage forms containing IBU, ten proprietary medicinal products marketed in Italy were examined by the methods described here. These products, analysed before their expiration date, confirmed the IBU content claimed on the label and no related substances were detectable. To detect UDP at trace levels, the extraction was carried out on more units of the same medicinal product and the extractive solution was concentrated.

The extractive procedure performed on equivalent amounts of the IBU reference standard excluded that the process could cause the drug degradation. Table 4 reports the UDP detected in the medicinal products and all the degradation compounds arising from the treatments described in Table 1. The interpretation of UDP mass

Table 3
Retention indices (I_x) and main ions of the MS spectra of Ibuprofen degradation products or their methyl derivatives acquired by GC-MS

Code[a]	MW[b]	Ion mass[c]	I_x[d]
IBUm	220	161, 177 119, 117, 91	1522
1m	236	193, 105, 133, 194, 134	1709
1d	250	207, 208, 148, 133, 105	1610
2	178	163, 43, 57, 178, 91	1379
2m	192	177, 178, 134, 161, 135	1322
3m	234	191, 192, 103, 175, 132	1712
4	176	161, 176, 134, 43, 91	1411
5m	192	133, 192, 105, 77, 51	1478
6m	192	159, 192, 149, 135, 91	1443
7d	222	163, 222, 131, 103, 59	1618
9m	164	105, 164, 77, 106, 79	1185
10	150	107, 150, 108, 77, 51	1287
11	134	134, 133, 91, 105, 77	1154
12	160	117, 160, 118, 115, 91	1228
43	176	147, 43, 91, 119, 76	1551
67m	178	163, 178, 147, 135, 103	1439

[a] m, monomethyl derivatives; d, dimethyl derivatives.
[b] MW, molecular weight.
[c] *m/z* of the five most intense ions, in descending order of intensity.
[d] Calculated as proposed by Van den Dool and Kratz.

spectra (Figs. 4 and 5) allowed to hypothesise the structures included in the possible degradation scheme of Fig. 3 and named in Table 2.

Compounds 9 and 11 were identified by comparing HPLC relative retention times, GC retention indices, mass and UV spectra with those of the market reference standards. The identification of the other UDP was confirmed using reference compounds obtained by simple and univocal textbook synthesis [14]. Compound 1, a known IBU metabolite [15], was synthesised by reducing the carbonyl group of compound 3 by sodium boronhydride. Compound 7 was yielded by submitting compound 5 to Cannizzaro reaction. The alcohol produced by dismutation, 2-[4-(hydroxymethyl)phenyl]propanoic acid, was identified by comparing its HPLC retention time (method A RRT = 0.052), GC retention index ($I_x = 1557$), UV ($\lambda_{max} = 219.5$ nm) and MS (135, 105, 194, 79, 91, 117 *m/z*) spectra with those of the substance obtained by submitting compound 5 to sodium boronhydride reduction. Compound 2 was obtained by sodium borohydride reduction of compound 4. Compound 12 was produced by dehydration of compound 2 under acidic conditions. Compound 10 was obtained from isobutylbenzene by direct sulfonation and alkaline fusion.

The outcomes shown in Table 4 deserve some comments. Compound 43, a known IBU degradation product [6] that in theory can arise from compounds 4 and 3, was not detected in either the forced treatments or the medicinal products. Compound 67 and UDP 9, 10, 11, 12, found in the products of the forced degradations, were not detected in the medicinal products examined. Noteworthy is the presence of compound 2, which also originates from photolytic degradation and which has shown toxic effects in vitro on erythrocytes and cultured fibroblasts [7]. Compound 8, hypothesised as parent of compound 10 in the scheme of Fig. 3, has never been detected in the forced degradations or in the medicinal products.

Different chromatographic methods were employed to exploit their different separative ability. This approach and the ensuing results warrant the following considerations. The HPLC method A employed derives from procedures reported in US and European pharmacopoeias, modifying for column size and introducing a linear gradient to allow the resolution of almost all analytes in acceptable time. With this method, only compounds 7 and 67 are not completely resolved (Fig. 2); it is possible, however, to improve their detectability by working at 240 nm and to assay compound 67 selectively at 260 nm. Moreover, while compounds 5, 7 and 67 can be separated and assayed using the HPLC method B, IBU and the other degradation products are, unfortunately, not detectable under these conditions.

The GC-MS method detects all IBU degradation products, most of them as methyl derivatives. Among volatile compounds (2, 4, 12, 43), only substance 2 required methyl derivatization to improve gas chromatographic performances (Fig. 1). As shown in Fig. 1, GC separation between the monomethyl derivatives of compounds 6 and 67 is difficult, even if selected ion monitoring (SIM) allows the selective assay of the monomethyl derivative of compound 6 using ion with $m/z =$ 150. However, these compounds can be assayed using the HPLC methods described above. The

Table 4
Degradation products detected in the forced degradations (from A to S) described in Table 1 and, at traces level, in some marketed medicinal products Ibuprofen containing (MP)

	Degradation products[a]												
	1	2	3	4	5	6	7	9	10	11	12	43	67
A	•	•	•	•	•	•	•						
B	•	•	•	•	•	•	•		•		•		•
C	•		•										•
D	•	•	•	•	•	•			•				
E			•	•		•							
F			•	•					•				
G	•		•	•	•	•	•						
H	•	•	•	•	•	•	•			•			
I			•	•	•								
L	•	•	•	•	•	•	•	•	•		•		•
M	•	•	•	•	•	•	•	•	•				•
N			•										
O	•	•	•	•	•		•		•				
P	•	•	•	•	•		•	•	•	•	•		
Q	•	•	•	•	•	•	•	•		•			
R	•	•	•	•	•	•	•	•	•	•			
S	•		•		•								
MP1	•		•	•	•		•						
MP2	•		•	•	•	•	•						
MP3	•	•	•	•	•		•						
MP4	•		•	•	•	•	•						
MP5	•	•	•	•	•		•						
MP6	•	•	•		•		•						
MP7	•		•	•	•		•						
MP8	•	•	•	•	•	•	•						
MP9	•	•	•	•	•	•	•						
MP10	•		•	•	•	•	•						

[a] As coded in Table 2.

peak overlap between the monomethyl derivative of compound 1 and the methyl derivative of compound 3, detectable by selectively monitoring ions 193 and 191, respectively, is an apparent problem that can be solved only through the exhaustive derivatization of compound 1. In fact, compound 1d is detectable also in the presence of compound 7d and it is possible to assay both using selected ions (1d $m/z = 207$; 7d $m/z = 163$).

To evaluate IBU degradation by GC it was necessary to study the performance of different methylation reagents on the degradation products. To date, this evaluation has been performed only for IBU [16]. Among the degradation products forming methyl derivatives, the molecules containing a benzylic group (compounds 1, 2) can give rise to not univocal reactions that also produce methyl ethers.

The study was developed using hydrogen chloride–methanol (HCl–MeOH), boron trifluoride–methanol (BF_3–MeOH) and diazomethane in diethyl ether (CH_2N_2) as methylating reagents and considering degradation products at 1% (w/w) of IBU concentration (0.3 mg ml^{-1}). Using HCl–MeOH 2 N at room temperature for 1 h [14], the methylation of the degradation products was incomplete or did not occur. At 60 °C for 20 min with HCl–MeOH 1 N the reaction for compounds 1 and 2 was not univocal. Under the same conditions, but using HCl–MeOH 2 N, only the methyl ether of compound 2 and the dimethyl derivative of compound 1 were produced. BF_3–

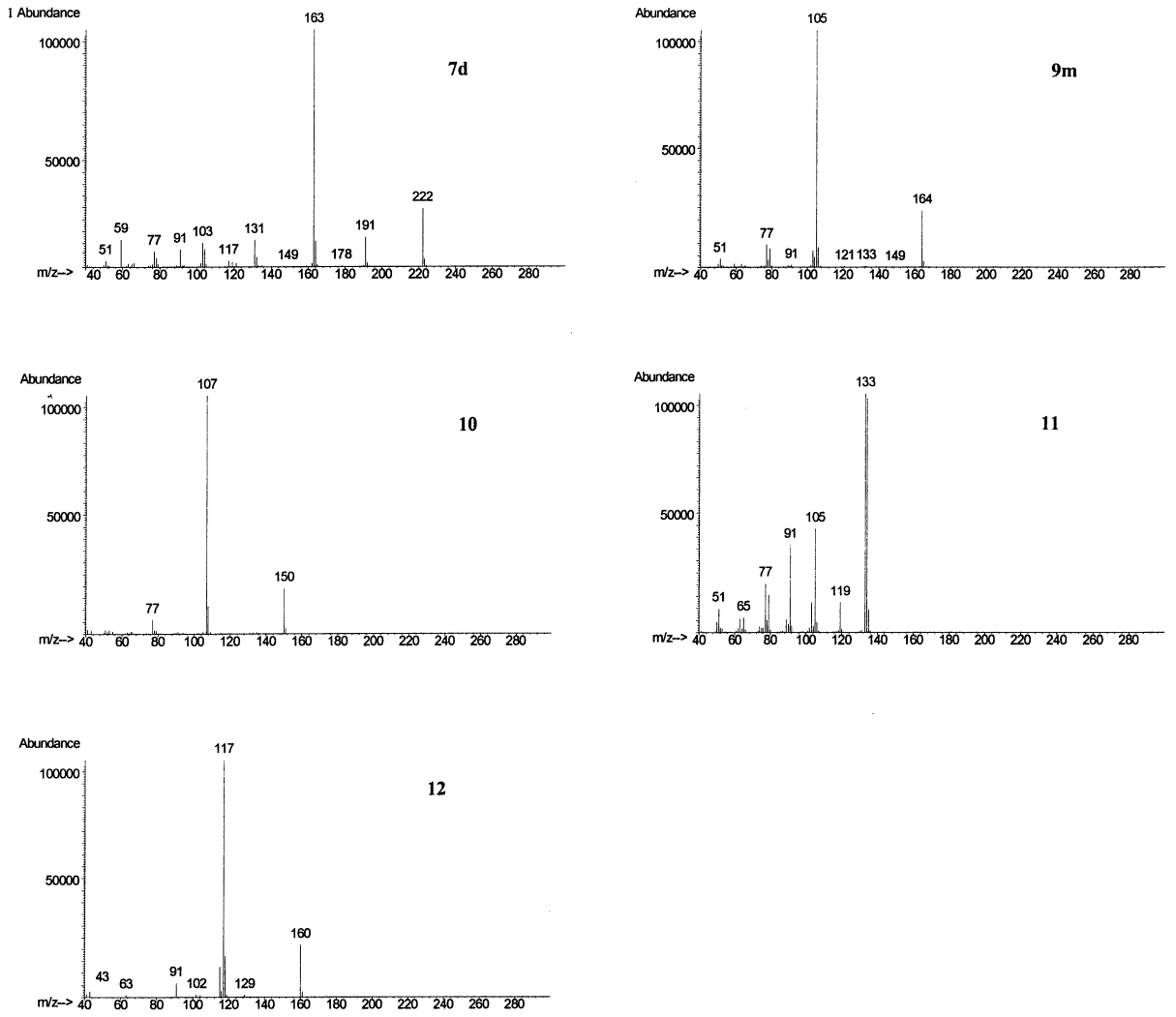

Fig. 4. Mass spectra of volatile (10, 11, 12) Ibuprofen degradation products, dimethyl esther of 7 (7d) and methyl esther of 9 (9m) (as coded in Table 2).

MeOH 14% (w/v), for 1 h at room temperature [14], methylated IBU but was not efficacious on substrates 1 and 2, even when their concentration was 0.05 mmol ml^{-1} and the reagent was used at the concentration of 50% (w/v). Heating at 60 °C for 30 min was necessary to obtain the full methylation of all degradation products at the lowest reagent concentration considered. CH_2N_2 in the described conditions did not methylate the alcoholic group of compounds 1 and 2, even when the concentration of these was 10% (w/w) of IBU. Under the same conditions only a trace amount of the methyl ether of compound 10 was yielded.

4. Conclusions

In this work, Ibuprofen was submitted to oxidative and thermal decomposition treatments.

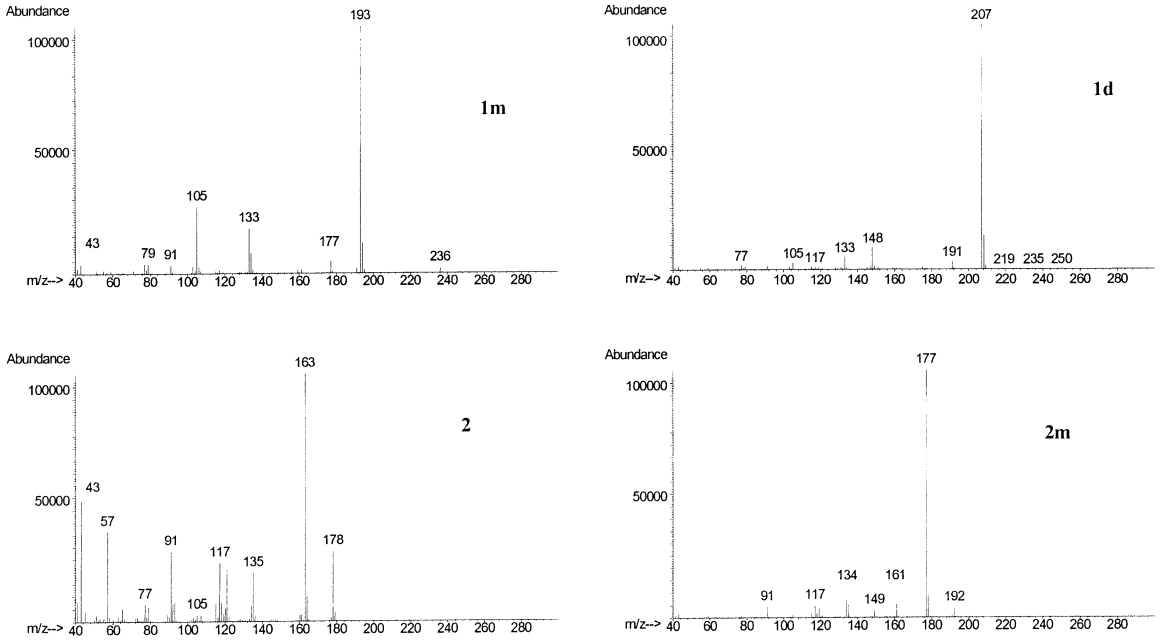

Fig. 5. Mass spectra of mono (1m) and dimethyl derivative (1d) of 1, of 2 and its methyl ether (2m) (as coded in Table 2).

Overall, 13 degradation products were detected. Seven of these compounds, which have never been related to the treatments described, were identified as Ibuprofen degradation products. The toxicological activity for one of these substances, 1-(4-isobutylphenyl)-1-ethanol, which is the parent molecule of the well-known toxic degradation product 4 isobutylacetophenone, has already been reported.

Because Ibuprofen is also a popular non-prescription drug that may entail prolonged use or abuse and considering its oxidative sensitivity, it is necessary to monitor the presence of all degradation products and to study their long term toxicity. The GC-MS and HPLC methods adopted in this study could be used for these purposes, given their ability to indicate Ibuprofen stability.

Acknowledgements

This work was partially supported by a grant from the Università degli Studi di Genova.

References

[1] J.E.F. Reynolds, Martindale, The Extra Pharmacopoeia, Royal Pharmaceutical Society, London, 1996, pp. 50–51.
[2] Goodman, Gilman's, The Pharmacological Basis of Therapeutics, 8th ed, Pergamon Press, New York, 1990, pp. 665–666.
[3] Medical Sciences Bulletin PIA 3, (1994).
[4] ECDIN data bank, Ispra, 1997.
[5] J. Zoeller (Ed.), American Druggist 2, 42–43 (1999).
[6] A. Dondoni, T. Dall'Occo, G. Fantin, A. Medici, P. Pedrini, V. Rossetti, Il Farmaco 7 (1986) 237–244.
[7] J.V. Castell, M.J. Gomez-L, M.A. Miranda, I.M. Morera, Photochem. Photobiol. 6 (1987) 991–996.
[8] F. Vargas, C. Rivas, M.A. Miranda, F. Bosca, Pharmazie 11 (1991) 767–771.
[9] P.A. Asmus, J. Chromatogr. 331 (1985) 169–176.
[10] European Pharmacopeia III ed., 1001–1002, EDQM, Strasbourg-F.
[11] J. Lee, H. Bertrand, D. Erkoboni, T. Finlay, H. Stamato, T. Wheatley, Pharm. Res. 9 (1995) S170.
[12] U.S. Pharmacopeia USP 24, Rockville, MD, 2000.
[13] Y. Sun, R. Zhang, Q. Wang, J. Chromatogr. A 657 (1993) 1–15.
[14] Voge's, Textbook of Practical Organic Chemistry, 5th ed, Longman Scientific and Technical, London, 1989.
[15] D.R. Kepp, U.G. Sidelmann, S.H. Hansen, Pharm. Res. 5 (1997) 676–680.
[16] L. Heikkinen, Acta Pharm. Fenn. 92 (1983) 275–282.