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Degradation profile and identification of the major degradation products of dobupride under several conditions by GC/MS and HPLC-particle beam/MS

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Abstract

The effect of pH, light, temperature and oxygen on the stability of dobupride (**1**), a novel gastroprokinetic drug, has been studied, storing the sample in the solid state and as a solution in methanol–water. The main forced degradation products have been identified by means of techniques such as GC/MS and HPLC-particle beam/MS, and two major degradation pathways have been characterized. One degradation route involves the loss of chlorine, yielding 4-amino-2-butoxy-*N*-[1-(1,3-dioxolan-2-ylmethyl)piperid-4-yl]benzamide (**4**) as the major degradation product. The second pathway results from cleavage of the piperidine–amide bond, producing 4-amino-2-butoxy-5-chlorobenzamide (**2**) as the major degradation product. Under the studied conditions, except when exposed to direct light in solution, dobupride has been shown to be very stable: after 5 months storage, the benzamide **2** (second pathway) was the only product identified (less than 0.5%). However, when dobupride in solution is exposed to natural or artificial sunlight, degradation is very fast, and after 7 days only 5% of the unchanged product remains. Under these circumstances, the main degradation route is the first one, with compound **4** being the most abundant degradation product, and compound **2** only being detectable in small amounts.

Keywords: Dobupride; Degradation products; HPLC/MS; Particle beam

1. Introduction

Dobupride (**1**, Fig. 1), 4-amino-2-butoxy-5-chloro-*N*-[1-(1, 3-dioxolan-2-ylmethyl)-piperid-4-yl]benzamide [1], is a new substituted benzamide drug which has shown powerful gastroprokinetic activity in animal studies, inducing gastric emptying and intestinal motility, and increasing the resting pressure of the lower oesophageal sphincter [2].

Different clinical trials have been carried out to study its activity in man [3,4]. Compared with metoclopramide, both drugs increased stomach emptying of liquid and also increased the lower oesophageal sphincter pressure, but after metoclopramide administration [3,4] more adverse effects (such as anxiety, drowsiness, and sweating) were reported.

In order to predict the stability of dobupride in different environments, the effects of several stress conditions (oxygen atmosphere, high temperature, pH, and direct light) have

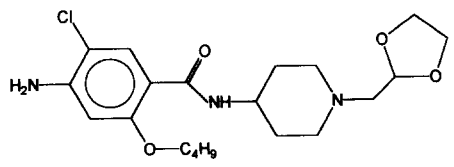

Fig. 1. Dobupride (**1**).

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been studied. Further, the degradation kinetic profile of dobupride under such conditions (as a solid, or in solution) has been established, and the major degradation products identified.

2. Experimental

2.1. Materials

Dobupride was from Almirall, S.A. (Spain). Methanol was HPLC solvent quality (Scharlau, Spain), and purified water was obtained from inverse osmosis followed by a Milli-Q (Millipore, USA) filtration. All the remaining chemicals were of analytical reagent grade: ammonium acetate, acetic acid, formic acid, and ammonium hydroxide (30%) were from Panreac (Spain), and tri-ethylamine from Scharlau (Spain).

2.2. HPLC Analysis

The system consisted of a HP 1050 chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with a quaternary pump, autoinjector and variable wavelength detector set at 254 nm. A Nova-Pak C_{18} 4-μm column (2 mm × 15 cm, Waters P/N 23655) was used for all analytical determinations. The mobile phase was methanol–0.1% triethylamine in water adjusted to pH 4.0 with formic acid (40:60, v/v) and was filtered and degassed prior to use. A flow rate of 0.4 ml min^{-1} produced a retention time of 17.7 min for dobupride. Peak areas were measured with a HP3396 Series II integrator. The injection volume was 5μl.

2.3. HPLC-particle Beam/MS

The HPLC system was attached by an HP59980A particle beam (PB) interface to an HP5988A mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA). The desolvation chamber temperature was set to 70 °C and the helium flow rate to 40 psi.

2.4. GC/MS

GC analysis was accomplished using a HP5890A gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) directly interfaced to a HP5988A mass spectrometer. The analytical column was a capillary HP-5 (cross-linked 5% phenyl methyl silicone gum phase, 15 m × 0.31 mm) column. The GC injection port temperature was 210 °C, and the inlet pressure of the helium carrier gas was maintained at 35 kPa throughout the chromatographic run. Injections were made in the splitless mode, and the injection port was purged 3 min after injection. The column temperature was initially set at 100 °C, and increased at a rate of 20 °C min^{-1}. The final temperature of 300 °C was held for 10 min. The GC/MS interface temperature was kept at 300 °C, and under these conditions the retention time for dobupride was 17.5 min.

2.5. Mass spectrometry

The GC/MS system components were controlled and data were acquired by the HP59970C computer system using HP MS Chemstation Pascal version 3.2 software (Hewlett Packard, Palo Alto, CA, USA). Data analysis were carried out using HP G1034C MS Chemstation (DOS-series) version C.00.07 software. The mass spectra were measured with both electron ionization (EI, at 70 eV) and positive ion chemical ionization (PCI, with methane, source pressure = 1 torr).

2.6. Stress conditions

Sample preparation

Two different series of samples of dobupride were prepared. One of them (solid state experiments) used quantities of about 20 mg of dobupride, exactly weighed, and transferred to a series of clear 10-ml volumetric flasks. These samples were distributed under several stress conditions as explained below, and analyzed at the end of each particular storage period, according to the storage protocol (Table 1). Just prior to the analysis all samples were dissolved in a methanol-purified water mixture (1:1, v/v), and made up to volume.

The other samples stored were solutions (dissolution experiments) of dobupride (2 mg ml^{-1}) in methanol–water (1:1, v/v).

The solid and solution samples of dobupride were maintained under different stress conditions as follows.

Temperature test

The samples were stored in the dark at 40 ± 2 °C and 80 ± 2 °C.

Table 1
Storage protocol

Stress parameter	Sample [a]	Protocol	Time unit
Temperature	A and B	0, 1, 2, 5	Months
Oxidation	A	0, 2, 5	Months
Sunlight	A	0, 1, 2, 5	Months
Sunlight	B	0, 2, 5, 7, 10, 12	Days
Suntest	A	0, 5, 10, 20, 40, 60, 80, 100	h
Suntest	B	0, 2, 4, 8, 12, 16, 20	h
pH	C	0, 1, 2, 5	Months

[a] A = solid sample; B = solution sample; C = Buffer–methanol solution sample.

Oxidation test

The head-space atmosphere of the flasks was purged with pure oxygen gas, then tightly crimped, and stored in the dark at room temperature.

Light test

(a) Sunlight. The samples were exposed to natural sunlight on a window sill. As a reference, one of the flasks containing dobupride solution, but protected from the light by wrapping with aluminium foil, was concurrently exposed to the same conditions over 12 days.

(b) Suntest. The forced photochemical degradation of the samples was also carried out in a SUNTEST (Heraeus, Germany) instrument, with 150 klux illumination, wavelength range from 300 to 800 nm, irradiation of 850 W m^{-2} and with air refrigeration (120 m^3 h^{-1}).

pH effect

The solution samples for this experiment were prepared as described under "Sample preparation" but using modified solvent with three different (1:1) mixtures of methanol with buffers. The buffer solutions used were 0.01 M ammonium acetate–acetic acid at pH 3.0; 0.05 M ammonium acetate–ammonium hydroxide at pH 7.0; and 0.01 M ammonium acetate–ammonium hydroxide at pH 10.0. These solutions were freshly prepared, and pH values of the aqueous buffer were measured by a pH meter (Microph 2000, Crison).

The buffer–methanol solution samples were analyzed by HPLC. No GC/MS analysis was carried out because of the salt content of these particular solutions.

3. Results and discussion

3.1. Identification of degradation products

LC-PBeam/MS has been successfully shown to be a very useful interface for the structural elucidation of impurities and degradation products of pharmaceutical drugs [5–9]. The generation of library-searchable, and GC/MS confrontable, EI and PCI spectra by HPLC-PB/MS offers an important advantage over other LC/MS techniques. For these reasons, the identification of the degradation products of dobupride has been carried out

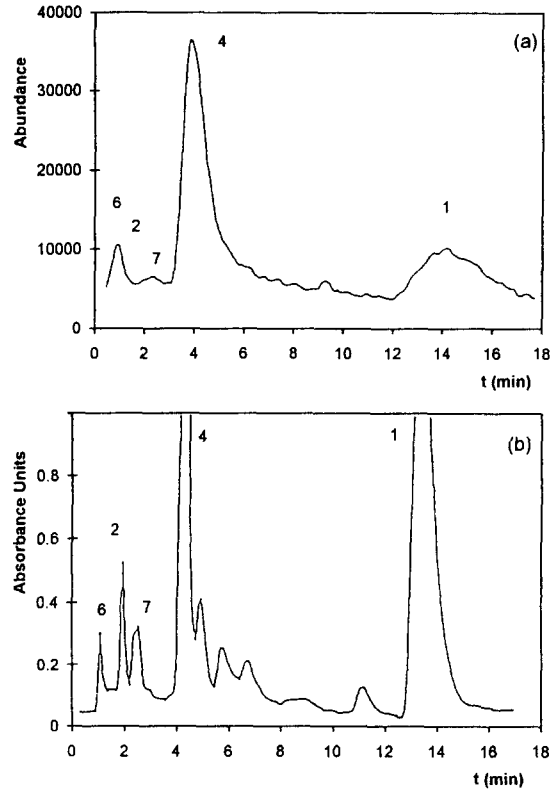

Fig. 2. 16 h of Suntest degradation in solution. (a) HPLC-PB/EI-MS chromatogram. (b) HPLC/UV chromatogram.

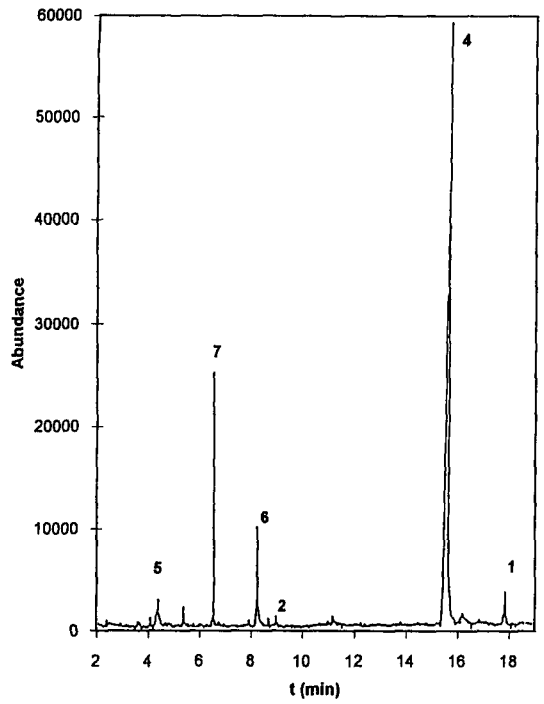

Fig. 3. GC/MS chromatogram for 7 days of sunlight degradation of dobupride in solution.

from the mass spectra (EI and PCI) obtained by both GC/MS and HPLC-PB/MS. In general, PB shows lower efficiency and sensitivity than other LC/MS interfaces (i.e. Fig. 2 shows the difference between HPLC/UV and HPLC-PB/MS chromatograms obtained from the same solution of dobupride degraded by Suntest), but is adequate to allow the detection of the degradation products and their further identification by EI and PCI spectroscopy. In this case, PB identification has been essential in order to demonstrate the selectivity of the HPLC/UV method, later used to carry out quantitative studies. In addition, full scan spectra of compound **2** can only be obtained by HPLC-PB/MS (compound **2** can only be detected by GC/MS using an SIM-selected ion monitoring-technique). In spite of compounds **2** and **6** having a low resolution by HPLC-PB/MS, as shown in Fig. 2, they can be easily deconvoluted using SIM techniques (the retention times observed after SIM analysis are slightly different).

Mass spectra of compounds **3** and **5** were only obtained by GC/MS. For the other compounds (**1**, **4**, **6** and **7**) there were no significant differences between GC/MS and HPLC-PB/MS spectra. Fig. 3 shows an example of a GC/MS (EI) chromatogram, corresponding to sunlight degraded dobupride solution. Dobupride (**1**) and the identified degradation products **2**, **4**, **5**, **6** and **7** are labelled.

Table 2 shows the GC and HPLC retention time, the molecular weights (PCI spectra) and the major fragments (EI spectra) of the degradation products obtained under different stress conditions (see Fig. 4 for compound structures).

The mass spectra of dobupride indicated a molecular ion at m/z 411 (and m/z 413 from the ^{37}Cl isotope) with very low abundance (9%). The most important fragmentations are shown in Fig. 5.

Compound **2** was identified as 2-butyloxy-4-amino-5-chlorobenzamide; the molecular ion at m/z 242/244, chlorinated and relatively abundant, suggested its structure. The base peak (m/z 169/171) was formed by the loss of a butoxy group.

The spectrum of compound **3** showed a very low molecular ion. However, its base peak (m/z 83) suggested a non-N-substituted piperidine. This compound was identified as *N*-(4-piperidyl)-2-butyloxy-4-amino-5-chlorobenzamide. The other fragmentations are the same as those of dobupride.

Compound **4** has a mass spectrum with the same fragmentations as dobupride, but all the peaks have 34 mass units less. This indicated the absence of chlorine in its structure and was identified as *N*-[1-(2-(1,3-dioxolanyl)methyl)-4-piperidyl]-2-butyloxy-4-aminobenzamide.

Compound **5** was idendified as 2-hydroxy-4-amino-5-chlorobenzamide; the molecular ion indicated the presence of chlorine. The most abundant fragments were m/z 70 $[CH{=}CH{-}/CONH_2]^+$ and m/z 44 $[CONH_2]^+$.

Compound **6** has a mass spectrum with the same fragmentations as compound **2**, but with all peaks without chlorine. Hence, it was identified as 2-hydroxy-4-amino-5-chlorobenzamide.

The molecular ion of compound **7** was very low mass, indicating that the product was not chlorinated. The base peak could be produced by the loss of the 1,3-dioxolanyl group (m/z 73 is normally produced by the 1,3-dioxolane ion).

3.2. *Effect of temperature, oxidation and pH*

The results obtained from different dobupride samples at several conditions of temperature, oxidation and pH in the dark, are collected in Table 3. They show that solid dobupride is only slightly affected by high temperatures, whereas in solution, although no degradation was detected after 5 months at 40 °C, around 3% was degraded at 80 °C. Similar degradation patterns were observed in the oxygen atmosphere and

Table 2
Mass spectra of degradation products [a]

Compound	HPLC Rt	GC Rt	MW	EI Fragments m/z (abundance %) [b]
1	13.3	17.6	411	226/228 (100/33), 170/172 (65/22), 338/340 (38/13), 411/413 (9/3) [b]
2	1.7	8.8	242	169/171 (100/33), 141/143 (26/9), 242/244 (24/8) 113 (10), 114 (9)
3	ND	11.9	325	83 (100), 170/172 (78/26), 226/228 (54/18), 241/243 (12/4), 325/327 (2/0)
4	4.1	15.3	377	192 (100), 136 (58), 304 (22), 377 (8)
5	ND	4.3	186	70 (100), 44 (75), 113 (60), 186/188 (15/5)
6	0.9	8.1	208	135 (100), 107 (45), 208 (24), 148 (24), 79 (20), 192 (10)
7	2.7	6.4	244	171 (100), 128 (33), 73 (15), 139 (14), 244 (6)

[a] Rt = retention time (min); MW = molecular weight obtained from PCI spectra; ND = not detected.
[b] The double values indicate the chlorine isotopes.

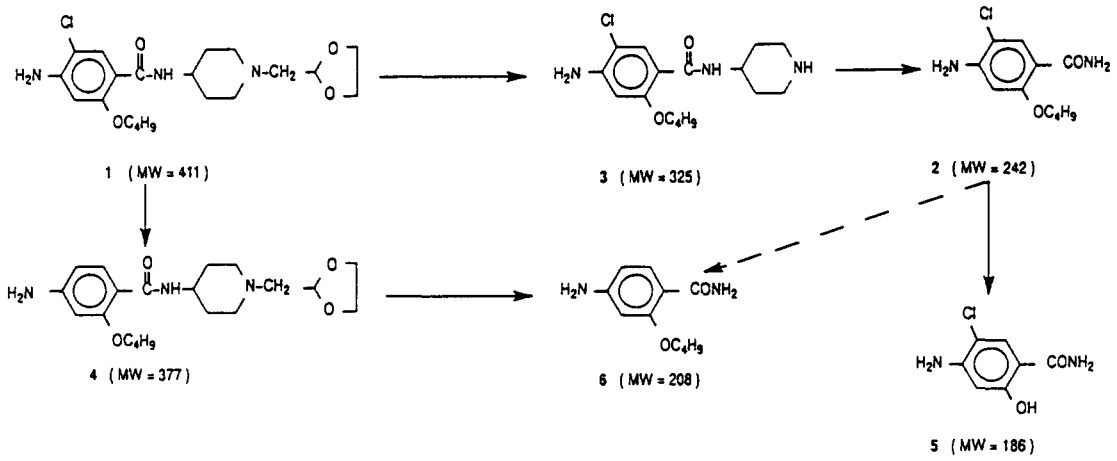

Fig. 4. General degradation pattern of dobupride (solid and solution).

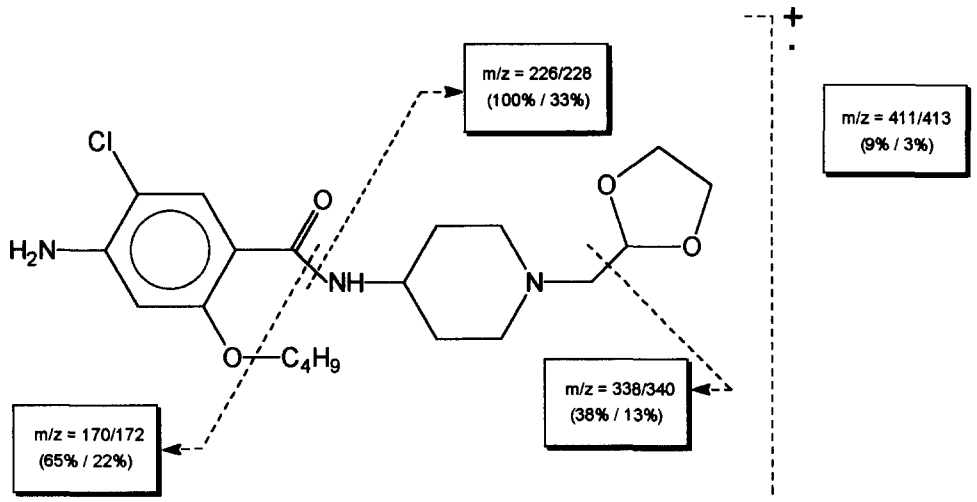

Fig. 5. Fragmentation pattern of dobupride by electronic impact. Abundances are indicated in brackets.

pH range experiments. The only identified degradation product was compound **2** ($\approx 0.5\%$). This compound results from the cleavage of the piperidine–amide bond.

3.3. *Effect of light*

The effect of natural sunlight on solid dobupride is very insignificant (remaining

Table 3
Stability of dobupride in the dark [a]

Time (months)	Analytical method	40 °C		80 °C		Oxy. atm.	pH 3	pH 7	pH 10
		A	B	A	B	A	C	C	C
1	HPLC/UV	100	100	100	99	100	100	100	100
2		100	100	100	98	100	100	100	100
5		99	100	99	97	99	99	99	99
1	GC/MS	100	100	100	100	100	–	–	–
2		100	100	100	100	100	–	–	–
5		100	100	100	99	100	–	–	–

[a] Expressed as unaltered dobupride (%) calculated by area normalization. A, B and C: same key as in Table 1.*

Table 4
Suntest degradation of dobupride in solid state

Technique	RRT [a]	%Area							
		0 h	5 h	10 h	20 h	40 h	60 h	80 h	100 h
GC/MS	0.22	0	0	0	0	0.08	0.09	0.14	0.17
	0.5 (2)	0	0	0	0.19	0.98	1.71	1.85	3.01
	0.57	0	0	0	0	0	0.28	0.22	0.36
	0.70	0	0	0	0	0	0	0.23	0.26
	1.00 (1)	100	100	100	99.81	98.94	97.93	97.56	96.21
HPLC/UV	0.10 (2)	0	0	0	0.1	1.4	2.16	2.49	4.09
	0.34	0	0.11	0.15	0.17	0.62	0.95	0.84	1.31
	0.61	0	0.15	0.17	0.19	0.72	0.83	1.05	1.39
	1.00 (1)	100	99.74	99.68	99.53	97.25	96.05	95.61	93.02
	1.83 (2)	0	0	0	0	0	0	0	0.19

[a] Retention time relative to dobupride. The identified compounds are indicated in brackets.

Table 5
Sunlight degradation of dobupride is solution

Technique	Product	% Area					
		0 days	2 days	5 days	7 days	10 days	12 days
GC/MS	1	100	40.9	7.3	4.9	0.71	0
	2	0	1	0.5	0.2	2.1	1.4
	4	0	53.8	84.6	78.1	60.6	48.8
	5	0	0.17	1	3.4	7.1	10.3
	6	0	1.5	2.1	4.9	8.3	11
	7	0	0.1	1.5	6.3	11.1	19.8
HPLC/UV	1	100	42.9	9.7	6.1	2.2	1.5
	2	0	2.1	5.9	7.6	6.9	6.8
	4	0	51.3	68.5	51.6	40.2	28.7
	6	0	1.1	6.2	15.3	23.1	25.1
	7	0	0	1.3	4.9	7.9	15.3
	Others	0	2.6	7.6	14.1	19.2	21.9

dobupride was 99.7% and 99.5% after exposures of 1 and 2 months, respectively). Because of its high stability to sunlight, more extreme conditions (Suntest) were applied. As Suntest produces intense and continuous lighting, degradation may be observed after shorter periods. Table 4 shows the results obtained by this test on solid dobupride, evaluated by GC/MS and HPLC/UV. Significant degradation started after 40 h exposure, and the most important degradation product was again compound **2**.

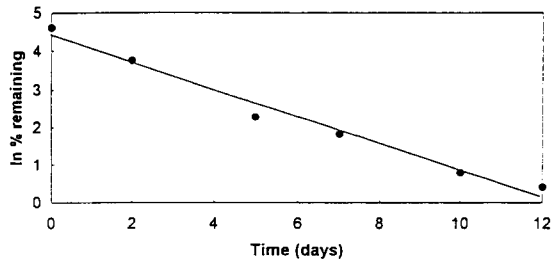

Fig. 6. Pseudo-first-order sunlight degradation kinetics of dobupride in solution analyzed by HPLC/UV.

The studies of the degradation of dobupride in solution were carried out in a methanol–water mixture owing to its low solubility in pure water. In this kind of sample preparation, dobupride proved to be more sensitive to light exposure. Under natural sunlight, degradation proceeded very rapidly (Table 5): after 2 days less than 50% dobupride remained and after 12 days only 1.9% was present in the sample (HPLC/UV detection).

To be sure that this high decomposition is only produced by light action, some samples were protected from light with aluminium foil. After 12 days, no degradation products were detected in these samples.

A typical semilogarithmic plot of the percentage of residual **1** versus time for the degradation kinetics of **1** in methanol–water solution under exposure to natural light is shown in Fig. 6. From the HPLC/UV data, statistical regression analysis gives excellent linearity ($r > 0.990$), which indicates that the degradation of **1** follows pseudo-first-order kinetics. The degradation rate constant (K_{obs}), calculated from the slope of the graph, is -0.35 per day and the half-life ($t_{1/2}$) is 1.37 days.

The dechlorinated compounds **4** and **6** are the major degradation products when dobupride is exposed to light in solution. Compound **2**, the major degradation product obtained under the other conditions, was only detected in smaller amounts, together with its derivative compound **5**.

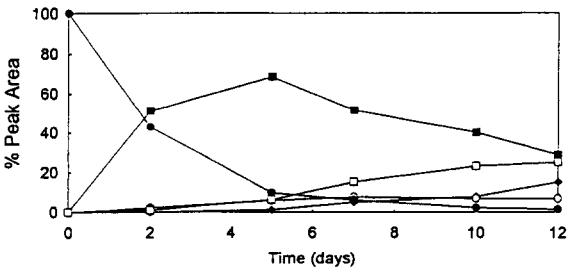

Fig. 7. Sunlight assay of dobupride in solution analyzed by HPLC/UV: **1** (●); **2** (○); **4** (■); **6** (□); **7** (◆).

The degradation profile of **1**, considering only the principal detected products, is shown in Fig. 7. Compound **4** reached a maximum concentration after 5 days, and when this compound started to decrease the other dechlorinated compound **6** became more abundant; this probably occurred (Fig. 4) by cleavage of the amide–piperidine bond, rather than from compound **2** by loss of chlorine.

The degradation profile of dobupride in methanol–water mixture by Suntest is very similar to that obtained by sunlight exposure. Only compound **3** was additionally identified by GC/MS in small amounts (about 0.8%). This compound could be an intermediate in the formation of **2**.

4. Conclusions

Dobupride has been shown to be very stable under the conditions studied, except when exposed to direct light in solution.

Depending on the degradation conditions and the type of sample preparation, two main degradation pathways have been characterized for dobupride, as represented in Fig. 4. When this molecule was in solution its immediate and major photochemical degradation product was deschloro dobupride (**4**).

In contrast, in the solid state and under exposure to high temperatures, light or oxygen, the main degradation product is **2**, resulting from cleavage of the piperidine–amide bond.

References

[1] A. Vega, J.M. Prieto, F. Pujol, J. Moragues and R.G.W. Spickett, European Patent 213775A1, 1987; Chem. Abstr., 106 (1987) 213581j.

[2] A.G. Fernández, J. Llupià, J. Puig, P. Berga and R. Massingham, Rev. Farmacol. Clin. Exp., 6 (1989) 257.

[3] M.U. Rashid and D.N. Batemean, Br. J. Clin. Pharmacol., 28 (1989) 748P.

[4] L. Grande, G. Lacima and A. Pérez, J. Clin. Nutr. Gastroenterol., 6 (1991) 18–22.

[5] V. Segarra, F. Carrera, J.L. Fábregas and J. Claramunt, Circ. Farmaceut., Special Issue — 4th Int. Symp. Pharmaceutical Sciences, Barcelona, 1991, p. 31.

[6] Apffel and M.L. Perry, J. Chromatogr., 554 (1991) 103–118.

[7] R.D. Voyksner, C.S. Smith and P.C. Knox, Biomed. Environ. Mass Spectrom., 19 (1990) 523–534.

[8] W.V. Ligon and S.B. Dorn, Anal. Chem., 62 (1990) 2573–2580.

[9] C. Julien-Larose, P. Voirin, C. Mas-Chamerlin and A. Dufour, J. Chromatogr., 562 (1991) 39–45.