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Slow, spontaneous degradation of lansoprazole, omeprazole and pantoprazole tablets: isolation and structural characterization of the toxic antioxidants 3H-benzimidazole-2-thiones

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The spontaneous degradation of lansoprazole, omeprazole and pantoprazole tablets upon long-term and forced storage conditions was determined by high performance liquid chromatography (HPLC). The more abundant products could be isolated by liquid chromatography and their molecular weights determined by Mass Spectrometry (MS). Their structures, established according to their spectroscopic data, were compared to those of either the literature or of authentic samples. Thus lansoprazole led mainly to a mixture of 3*H*-benzimidazole-2-thione (**2a)** and 3*H*-benzimidazole-2-one (**2c)**, omeprazole mainly to a mixture of 5-methoxy-3*H*-benzimidazole-2-thione (**1a)** and 2-hydroxymethyl-3, 5-dimethyl-4-methoxypyridine (**1b)**, and pantoprazole, to 5-difluoromethoxy-*3H*-benzimidazole-2-thione (**3a)** and 2-hydroxymethyl-3, 4-dimethoxypyridine (**3b)**. Although some of the degradation products had already been observed under different conditions, the detection of benzimidazole-2-thiones is unprecedented and their involvement as possible physiological, yet toxic antioxidants must be emphasized. Plausible, unified mechanisms for the formation of the different degradation products observed herein and in previous papers from the literature are suggested.

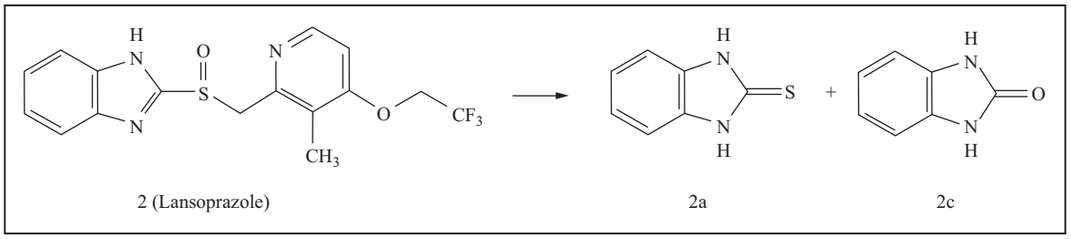
1. Introduction

Lansoprazole, omeprazole and pantoprazole are [(pyridylmethyl) sulfinyl] benzimidazoles, so-called proton-pump inhibitors, known for their high activity as gastric (H^+, K^+)-ATPase inhibitors. [(Pyridylmethyl) sulfinyl] benzimidazoles possess long-lasting acid antisecretory properties associated with anti-ulcer and anti-gastroesophageal reflux activities (Brandstrom et al. 1989; Lindberg et al. 1986; Sachs et al 1995; Krüger et al 1990; Rackür et al 1985; Hogg 1990)**.** They are also known for their effectiveness in the Zollinger-Ellison syndrome (Del Valle et al. 2003). More recently, their antioxidant properties have also been put forth in efforts to explain their role in the gastro-protection towards radical species (Kohler et al. 2010; Biswas et al. 2003; Becker et al. 2006; Natale et al. 2004; Koch et al. 2004; Lapenna et al. 1996; Backlund et al. 1999). Yet, no clear-cut structure-related arguments in favour of theses properties have been given, their antioxidant properties being possibly either inherent to the drugs themselves or to some of their degradation products: Lansoprazole, Omeprazole like benzimidazole and 2-mercaptobenzimidazole interact with the cytochrome P-450 system in the liver and inhibit several liver-monooxygenase activities. These compounds appeared to be rather unstable with respect to external factors such as acids (Stroyer et al. 2006). Efforts have been made to improve their stability by modifying for example the substituents in the pyridine and in the benzimidazole moieties so as to obtain compounds with both high (H^+, K^+)-ATPase inhibitory activity in stimulated gastric glands, but low reactivity (high chemical stability) at neutral pH.

In vitro investigations on the mechanism of action of these compounds have demonstrated that they are indeed pro-drugs which are transformed under physiological conditions into cyclic sulfenamides the pharmacologically active intermediates (Krüger et al. 1990; Hogg 1990).

As a result of their inherent instability, it was mandatory to determine the presence of impurities in the final pharmaceutical ingredients and to establish their structure. Although the degradation of lansoprazole, omeprazole and pantoprazole had already been described under various conditions, the exact structure of the newly formed degradation products had up to now not been determined (EL-Sherif et al. 2006; USP 2004). Several papers devoted to the analytical data on these drugs and describing, on the one hand, the possible presence of process-related impurities, originating directly from the batches, appeared in the literature (Reddy et al. 2007; Ph. Eur 7.6 2013). On the other hand omeprazole has recently been observed in surface waters together with some degradation products, *eg* the toxic 2-hydroxymethylpyridines (Apollo Scientific Ltd, Safety Data Sheet 2005), resulting, according to these authors, from its interaction with water and acids (Della Greca et al. 2006).

However, to the best of our knowledge, no quantitative data on the direct, *per se*, slow, long term transformation of these compounds, as raw material or in the form of enteric-coated tablets can be found in the literature. Therefore we carried out



Scheme 1: Degradation products of lansoprazole 2.

stress studies on these blister-protected pharmaceuticals in order to establish their inherent stability (according to ICH guidelines) and to identify their possible degradation products.

The aim of this paper is first to confirm that indeed a slow degradation of a series of proton-pump inhibitors, lansoprazole, omeprazole, and pantoprazole takes place over time, both under regular (25 °C, 60% room humidity (RH)), and under accelerated storage conditions (40 °C, 75% RH); second to isolate and determine the structure of the most abundant degradation products. As it turned out, the results described herein differ mainly from previous observations of the literature in that, in all the cases examined, 3*H*-benzimidazole-2-thiones **1a, 2a, 3a**, among others, were detected as degradation products. The presence in the proton-pump inhibitors of even tiny amounts of these compounds, the antioxidant properties of which are well established, might possibly account for their observed acid-independent protective effect during various gastric mucosal damage conditions. Reversely, the known adverse effects of these degradation products (potent thyroid toxicity) should be addressed and care should be taken in order to limit this degradation process (Gaorski et al. 1991; Kawasaki et al. 1998; Sakemi et al. 2002).

2. Investigations and results

Samples of the various commercially available products were placed in a climatic chamber (Angelantini, model CH1500) under regular storage conditions (25 °C, 60% RH, for three years) or under accelerated conditions (40 °C, 75% RH, for eight months). The chemical analyses of successive samples were performed respectively either after each 6 months or monthly, using HPLC.

2.1. Degradation products of lansoprazole

In the case of lansoprazole tablets**,** the purity of the starting tablets was determined by HPLC: only lansoprazole (**2**) could be detected.

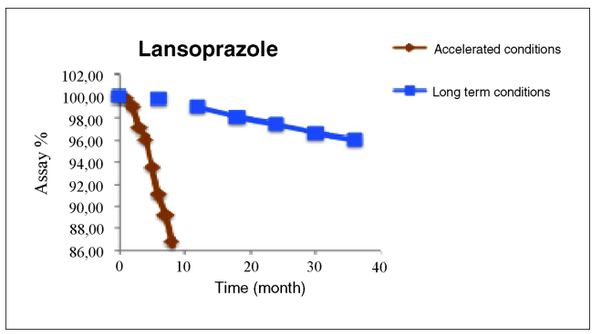


Fig. 1: Decrease of the amount of lansoprazole under long-term regular conditions (25 °C, 60% RH) and under accelerated conditions (40 °C, 75% RH).

Under long-term regular conditions (25 °C, 60% humidity), the enteric-protected tablets of lansoprazole underwent a slow transformation, already detectable by HPLC after a short period of time, giving seven compounds, the most abundant degradation product having the lowest retention time. Under accelerated conditions (40 °C, 75% humidity), such tablets led much faster to a mixture of the same products. The decrease of the amount of lansoprazole versus time, determined by HPLC is shown in (Fig. 1).

The more abundant degradation products were isolated *via* successive column and thin- layer chromatography as described above, and their purity checked again by HPLC, giving two well-separated peaks, with retention times of 0.46 and 0.92 minutes. The first product **2c,** (m/z = 135), a white solid with m.p. > 300 °C, exhibited a very simple ^{1}H NMR spectrum in DMSO, showing two series of signals, at respectively δ10.69 ppm attributable to NH protons, and at δ6.94, attributable to aromatic protons.

The ^{13}C NMR spectrum, disclosed four signals at δ 155.89, 129.88, 120.75, and 108.73 ppm. All these data, together with the elemental analysis, agreed with structure **2c,** thus with the formation of 3*H*-benzimidazole-2-one.

To the more retained product, isolated as a high-melting solid, was given structure **2a**. The elemental analysis confirmed the presence of sulphur and agreed with the formula $C_7H_6N_2S$ (Table 1) as did the electro- spray mass spectrum ($M^+Na = 173.014$).

The ^{13}C NMR spectrum of **2a** was very close to the spectrum of **2c**, with signals at δ168.33, 132.66, 122.57, and 109.95 ppm.

The ^{1}H NMR spectrum disclosed the expected signals at δ12.52 (NH) and at δ 7.11 ppm. Altogether, these data matched with the structure **2a**, (Scheme 1) (Sutter et al. 1999).

2.2. Degradation products of omeprazole

In the case of Omeprazole tablets**,** the purity of the starting tablets was again determined by HPLC.

Under long-term regular conditions, the enteric-protected tablets of omeprazole led to seven compounds, the most abundant having the lowest retention time. At higher temperature, such tablets led much faster to a mixture of the same products. The decrease of the amount of Omeprazole versus time, determined by HPLC is shown in (Fig. 2). The more abundant degradation products (retention times 0.54 and 0.94 minutes) were isolated as for lansoprazole.

The ESI mass spectrum as well as the elemental analysis of the first product, which clearly indicated the presence of sulphur agreed with those of 5-methoxy-3*H*-benzimidazole-2-thione (**1a**), a result confirmed by the NMR data of the isolated compound. Thus, the ^{1}H NMR spectrum depicted signals at δ 12.42 (m, 2H), 7.08 (m, 1H), 6.72 (m, 2H) and 3.80 (s, 3H) ppm**.** The ^{13}C NMR spectrum showed signals at δ 168.01, 156.14, 133.44, 126.75, 110.42, 109.92, 94.86 and 55.83 ppm.

All these data together with the melting point, 255 °C, were in agreement with literature values for **1a**. Structure **1b** was

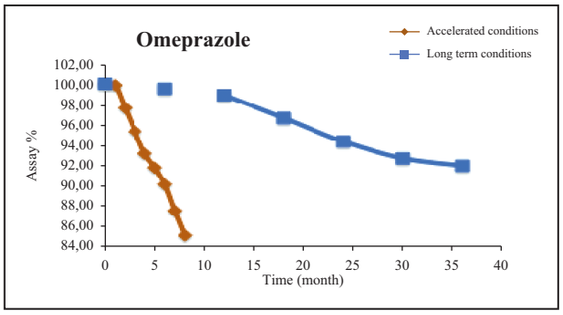


Fig. 2: Decrease of the amount of omeprazole under long term regular conditions (25 °C, 60% RH) and under accelerated conditions (40 °C, 75% RH).

assigned to the second, more retained product on the following grounds: the ESI mass spectrum was in accordance with such a structure. The elemental analysis confirmed the molecular formula together with the presence of nitrogen. The ^{1}H NMR spectrum disclosed a series of expected signals at δ 7.87 (1H, H^{6}), 5.07 (1H, OH), 4.41 (2H, H^{a}), 3.50 (3H, H^{d}), 2.00 (6H, H^{c} and H^{d}). The ^{13}C NMR spectrum, showing up signals at δ 163.74 (C^{4}), 156.56 (C^{2}), 147.39 (C^{6}), 124.83 (C^{5}), 122.57 (C^{3}), 61.67 (C^{b}), 9.20 (C^{d}) ppm confirmed these assignments. All these data together with the melting point, (55-60 °C), were again in agreement with those of the literature for **1b**. Scheme 2 features the degradation of omeprazole.

2.3. Degradation products of pantoprazole

In the case of pantoprazole tablets**,** the purity of the starting tablets was again determined by HPLC: only pantoprazole (**3)** could be detected.

As for the previous compounds, time-dependent degradation of pantoprazole was observed, the degradation being again faster at higher temperature and leading to a more complex mixture of products (presence of four extra products), most of them being however the same as those obtained under long-term, regular conditions. The decrease of the amount of Pantoprazole versus time, determined by HPLC is shown in Fig. 3.

Two of the more abundant products could again be separated and isolated and their structures fully established (Scheme 3).

A first product **3a** (R_t 0.52 mn) obtained as a solid, m.p. 290 °C, contained again sulphur according to the elemental analysis (Table 1) and agreed with the molecular formula $C_8H_6F_2N_2OS$. The ^{1}H NMR spectrum disclosed signals at δ 12.69 ppm (2 NH), at δ 7.17 ppm, as a triplet $J = 74$ Hz, typical, according to the literature for the CHF_2 proton observed for example for pantoprazole, at δ 7.20 (d, 1H, $J = 2$ Hz) and $\delta = 6.89$ (m, 2H) ppm for the aromatic protons. The ^{13}C NMR spectrum exhibited a series of signals which, according to a DEPT sequence, are quaternary

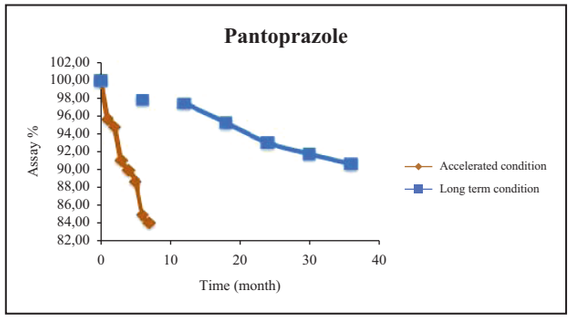


Fig. 3: Decrease (%) of pantoprazole in the tablets with time, under long-term conditions (25 °C, 60% RH) and under accelerated conditions (40 °C, 75% RH).

carbons, at δ 170.31, 147.42, 133.82, and 130.90 ppm, and at δ 117.78 (t, $J = 74$ Hz), 115.22, 111.74 and 101.30 ppm for four tertiary carbons, among which figures the signal for the carbon of the CHF_2 group, giving a typical triplet with a large coupling constant (J = 257Hz).

All these data matched thus with a structure such as **3a**, the known 2-difluoro-2-benzimidazole-thione. To the second product (R_t 1.56 mn), isolated as a white solid, m.p. 85 °C, was given structure **3b** on the following grounds. Its mass spectrum agreed with the molecular formula $C_8H_{11}NO_3$.

The proton NMR spectrum disclosed typical signals for two adjacent protons of a substituted pyridine, at δ 8.17 (d, $J = 5$ Hz) and 7.05 (d, $J = 5$ Hz) ppm, for two protons of a CH_2OH group, at δ 4.52 ppm, as a singlet, and for the protons of two methoxy groups at δ 3.87 and 3.75 ppm.

The ^{13}C NMR spectrum disclosed signals at δ 158.31, 152.75, 144.79, 141.91 and 106.74 ppm for the five aromatic carbons, together with signals at δ 60.69, δ 59.49 and δ 55.50 ppm for the carbons of the two methoxy groups and the carbon of the hydroxymethyl group.

3. Discussion

The *in vivo* and *in vitro* transformations of [(pyridylmethyl) sulfinyl] benzimidazoles has been the matter of numerous studies. Early reports clearly demonstrated that under physiological *highly acidic conditions*, these substrates underwent fast and deep transformations, giving *inter alia* the real active principle originating from a sulfenic acid.

In contrast, experiments conducted at higher pH indicated that the drugs were quite stable. The ***presence*** of impurities in the laboratory batches, or even in the finished drugs *eg* starting materials or their derivatives such as **1a, 2a, 2c** and **3a** (Ph.Eu.7.5) and side-reaction products, has also been disclosed (Reddy et al. 2007). Finally, the abiotic transformations of these substrates in an aquatic environment has been outlined, confirming that water, in the absence of any other reagent, was able to induce the degradation of omeprazole and lansoprazole into the benzimidazolones **2a** and more surprisingly, into benzimidazoles **5** and hydroxymethylpyridines **1b**.

Moreover, up to now, the striking *formation* of benzimidazole-2-thiones **1a**, **2a** and **3a** from the drugs 1-3, besides benzimidazole-2-ones **2c**, and to a lesser extend, of hydroxymethylpyridines **1b** and **3b** in all the cases examined herein, had never been observed before.

Since no acid is present in the commercially available tablets, it seems clear that the transformations observed under non-physiological conditions must be different from a mechanistic point of view. It has been shown that in the final product, obtained on an industrial scale, water is always present; therefore only water would possibly be able to interact either with the starting substrates or with their degradation products (Della Greca 2006). According to the structure of **1b** and **3b**, the main degradation products observed herein, it is clear that the sulfur-carbon bond, which links the sulfur atom to the methylpyridine ring, has been cleaved, and that a hydration reaction took place.

Evidence for the existence of a week carbon-sulfur bond in lansoprazole came from the examination of its MS/MS spectrum (Fig. 4). It is indeed known that fragmentation of a molecule is virtually absent in electrospray mass spectrometry, but that it can be easily induced by MS/MS technique which employs collision-induced dissociation to fragment a precursor ion.

3.1. ESI - MS analysis of lansoprazole

In methanol solvent, the negative ion spectra from 10 μM solution of lansoprazole revealed a prominent molecular anion for

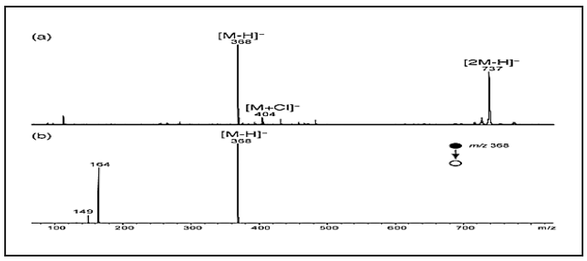


Fig. 4: (a) ESI spectrum of lansoprazole recorded in the negative ion mode and (b) CID spectrum of the $[M-H]^-$ ion m/z 368.

the singly charged molecule at *m/z* 368.0. The molecular mass of the uncharged lansoprazole is 369.076, and the signal at m/z 368 represents the deprotonated Lansoprazole. In addition, $[M^+Cl]^-$ anion is detected at *m/z* 404 and the non-covalent deprotonated dimer $[2M-H]^-$ is detected at *m/z* 737.

3.2. MS/MS of lansoprazole

The Collision Induced Dissociation (CID) spectrum of lansoprazole deprotonated molecule showed a single, prominent product ion at *m/z* 164 (plus a weak fragment at *m/z* 149). The calculated *m/z* values of the proposed structures for these ions were in acceptable agreement with the measured values.

Cleavage of the carbon-sulfur bond which links the benzimidazole ring to the pyridine ring took therefore place. This fragmentation process results in the formation of A and B, A being a stable radical anion. (Scheme 4). Omeprazole and pantoprazole behaved similarly. Having established the position of the weakest bond in lansoprazole, one can speculate about a mechanism accounting for the slow transformation of lansoprazole, omeprazole and pantoprazole under long term, non-acidic conditions (Scheme 5). These drugs contain two nucleophilic centers able to react with the electrophilc carbon - nitrogen double bond: the nitrogen atom of the pyridine and the oxygen atom of the sulfoxide. In the first case, under acidic conditions, such an interaction leads to the physiological active species of the drugs. The second case, **(Scheme 5, 1-3 → 7)** might be related, at least in its first steps, to the Moffatt oxidation of alcohols into ketones (Moffatt 1971), which involves indeed the interaction of a sulfoxide, dimethylsulfoxide, with a carbon-nitrogen double bond of an imide, dicyclohexylcarbodiimide. Applied to the case of lansoprazole, such a concerted, intramolecular nucleophilic addition, followed by a deprotonation reaction involving the oxygen atom, the carbon-nitrogen double bond and a hydrogen atom of the methylene group in **1-3**, might lead to **7**. The first step is likely to be a reversible process with the equilibrium lying far on the side of the starting drugs **1-3**. We hypothesized therefore that the evolution of such a key-intermediate **7** might lead to all of the observed products (Scheme 5).

A multistep cleavage of the carbon-sulfur bond in **7** might lead via the carbenoid species **9** to the hydroxymethylpyridines **1b** or **3b** upon a hydration step and to thiooxiranes **8. 8** could evolve *via* three routes **I, II and III**. Extrusion of either oxygen *(via* **I***)*, or sulfur (*via* **II**), or SO (*via* **III**) would lead respectively to the observed benzimidazole-2-thiones **1a, 2a or 3a,** the benzimidazole-2-ones **2c** and to the benzimidazoles **5,** the formation of which had indeed been observed by Della Greca and coworkers (Sutter et al 1999). Taken together, the slow formation of all of the observed products might be explained, *on a formal point of view*, by the involvement and evolution of pseudo-carbenoid species (Gaorski et al (1991) resulting from an intramolecular self-degradation of the [(pyridylmethyl) sulfinyl] benzimidazoles **1-3**.

These slow degradation reactions, though interesting on a chemical point of view, yet raise the question of the health impact of the formed products. Although it has been established that the acute toxicity of 2-mercaptobenzimidazole (*eg tyrotoxicity in rats*), a rubber-stabilizer and corrosion-inhibitor antioxidant (Kawasaki et al 1998; Sakemi et al 2002; Xu et al 1995) is rather low, its potent toxicity has been demonstrated. Moreover, it is a fat-soluble compound, which might also act as an endocrine disrupter. Since it has been recommended that the contamination level by such compounds should be reduced as far as possible, care should thus be taken to limit their amount in the drugs administered over long period of times. This might be achieved in the case of the proton-pump inhibitors by a strict observation of the stocking conditions.

3.4. Conclusion

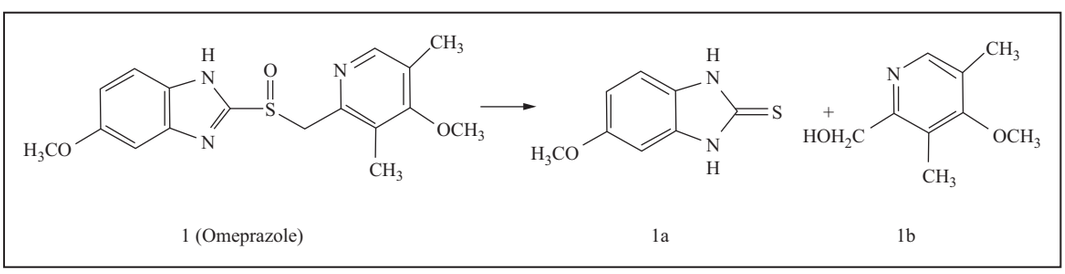
A complex, slow transformation of lansoprazole, omeprazole and pantoprazole tablets under normal and accelerated conditions has been observed: it takes place even at room temperature and is accelerated at higher temperature, lansoprazole showing the highest stability. Among the products isolated by liquid chromatography and characterized by spectroscopic methods, the three corresponding benzimidazole-2-thiones **1a, 2a and 3a** could be identified as products of spontaneous decomposition for the first time. The mechanism of formation of these degradation products is probably not related to the mechanism of formation of the physiological active intermediates, but is due to a slow intramolecular degradation process.

4. Experimental

4.1. Samples and chemicals

The standard samples of lansoprazole, omeprazole, and pantoprazole, were obtained from Sl Drugs and Pharmaceuticals. Their purity was established as 99.93, 99.97 and 99.91 % respectively. The investigated commercially available sample of Omepral (40 mg capsules) were obtained from Asia Pharmaceutical Industries (Aleppo, Syria). Commercially available Duogaste (30 mg capsules) and Progast tablets containing 45.104 mg of sodium pantoprazole (equivalent to 40 mg of pantoprazole), were obtained from Kaspar-Chabani Pharma (Aleppo, Syria) in the form of enteric-coated tablets.

Triethylamine, potassium hydroxide, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, silica gel 60 (0.063–0.200 mm) and silica



1 (Omeprazole) 1a 1b

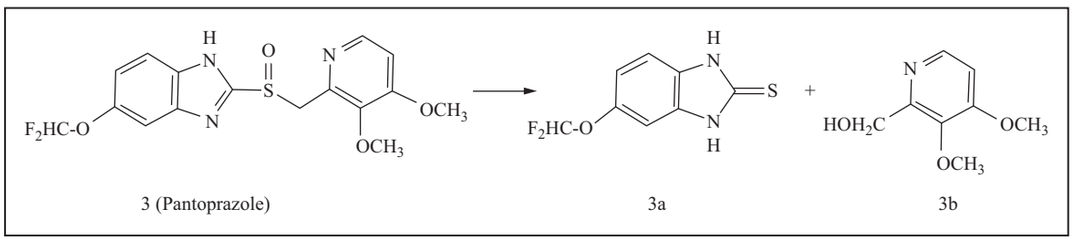
Scheme 2: Degradation products of omeprazole 1.

gel 60 F_{254} (0.063–0.200 mm) were obtained from Merck. Dichloromethane, isopropanol, and acetonitrile were obtained from LAB-SCAN (Ireland).

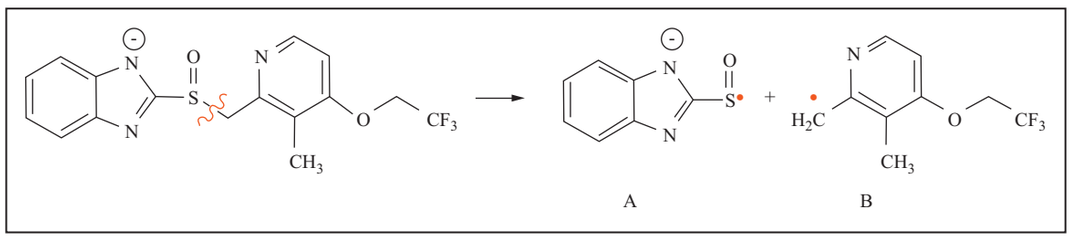
4.2. Chromatography methods

A Liquid Column Chromatography (LC) gradient method was developed for the separation and the determination of the purity of the isolated degradation products. An Agilent (model 1100, USA) HPLC apparatus was used. The buffer solution used for the preparation of the mobile phase was prepared from KH_2PO_4 (3.00 g) and K_2HPO_4 dissolved in water (1000 ml). The pH was adjusted to 7.4 with KOH (1N). The mobile phase was prepared by mixing 1000 ml of the previously prepared buffer with acetonitrile (500 ml). The pH of the solution was adjusted to 7.4 using KOH (1N). Three samples of the pharmaceutical standard drugs (0.1 mg/ml) were prepared together with three samples of the degraded samples. The samples were injected in the HPLC column and the percentage of the active substance was calculated by measuring the areas of the samples with respect to the areas of the standard.

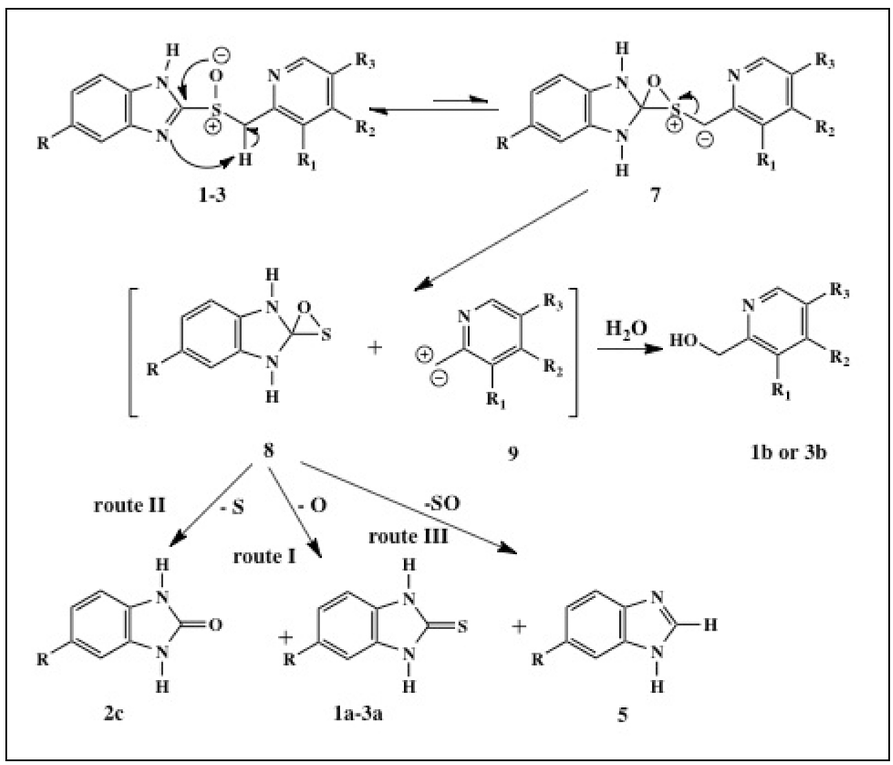
The degradation products of the various samples were isolated by using LC. The column was filled with a suspension of silica gel in methanol. The following mobile phase, obtained by mixing: dichloromethane/isopropanol/triethylamine (80/20/0.5) was then used for the separation of the different degradation products from the starting samples. The samples were prepared in the following way: the product obtained after storage either under normal or accelerated conditions in the form of blister-protected tablets was grinded and the powder was thoroughly extracted with methanol with vigorous shaking; the suspension was then filtered, and the filtrate submitted to LC. The various new compounds were separated according to their polarity by using the prepared mobile phase. Samples of the collected fractions were checked by TLC. A final purification of the isolated products was carried out by using preparative thin layer chromatography and acetonitrile as the eluant. Finally the purity of the isolated



Scheme 3: Degradation products of pantoprazole 3.



Scheme 4: MS/MS sulfur carbone bond cleavage in lansoprazole.



Scheme 5: Suggested mechanism.

compounds was determined by HPLC, by using a DAD UV detector. The new analytical method has been validated for Lansoprazole, Omeprazole and Pantoprazole by accuracy, precision, range, linearity, specificity/selectivity, limit of detection (LOD) and limit of quantitative (LOQ) tests.
The selectivity of the HPLC method was illustrated in their chromatograms, where complete separation of lansoprazole, omeprazole and pantoprazole from their degradation products were noticed with sharp peak and clear baseline separation. Linear correlation was obtained between the peaks areas and the corresponding concentrations for lansoprazole, omeprazole and pantoprazole in the range of 2- 3 mg/100 mL. The regression equations were calculated. The accuracy of the proposed method was checked by analysing different concentrations of the drugs in pure powdered form. The results indicate that the accuracy of the proposed method is not affected by the presence of excipients. The proposed HPLC method was successfully applied for the determination of these drugs in their formulations (blister protected tablets and powders).

4.3. NMR spectroscopy

The ^{1}H and ^{13}C NMR spectra and DEPT (Distortion Enhancement by Polarization Transfer) experiments for the different isolated compounds were carried out at 100 and 400 MHz on Brucker AVANCE spectrometers either in DMSO-D_6 or in methanol-D_3. The ^{1}H chemical shift values were reported on the δ scale in ppm, relative to TMS, and the chemical shift values were reported relative to $CDCl_3$ ($\delta = 77.0$ ppm) and DMSO-D_6 ($\delta = 30.50$ ppm) as internal standards, respectively.

4.4. Mass spectrometry

The analysis was performed on a SHIMADZU model LC/MS-2010 2V mass spectrometer. High-resolution positive and negative ESI mass spectra were acquired with a recent ultra-high resolution mass spectrometer, the hybrid linear ion trap LTQ-Orbitrap-XL (Thermo Fisher Scientific, Les Ulis, France). The analyses were performed by flow-injection analysis using a LC pump. Each direct introduction analysis was carried out by injecting 20 μL sample within a flow rate of 100 μL/min of a mobile phase consisting of methanol (around 100 ng of sample were injected). The electrospray voltage was set to 4.5 kV, the capillary voltage and the tube lens offset were set to 20 V and 70 V, respectively. The sheath and auxiliary gas flows (both nitrogen) were optimized at 40 and 15 (arbitrary units) and the drying gas temperature was set to 250 °C. The mass resolving power (full width at half maximum) was set at 6.10^4 FWHM at m/z 400. Tandem mass spectrometry was carried out with an electrospray-quadrupole ion trap mass spectrometer (Esquire 3000, Bruker, Bremen, Germany) operated in the negative ion mode. Samples (10 μL in MeOH) were directly infused using a syringe pump with a flow rate of 120 μL/min. Sequential MS^n experiments have been carried out with a Low Mass Cut Off (LMCO) corresponding to 28% of the *m/z* of the precursor ion ($q_z = 0{,}25$).

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