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Photostability and phototoxicity studies on diltiazem

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Abstract

The photostability of diltiazem was studied in aqueous solutions at different pH values. Firstly, the hydrolysis of the drug to desacetyldiltiazem in alkaline medium was evaluated and then the drug photodegradation under exposure to UVA–UVB radiation (solar simulator) was monitored by HPLC methods. The main photoproduct was isolated and characterized as diltiazem-*S*-oxide on the basis of the NMR and mass spectra. The HPLC method was also applied to the selective analysis of diltiazem in commercial formulations. Tests on mutagenicity and photomutagenicity of the drug were also carried out using *Salmonella typhimurium* TA 102 strain. In this testing the drug neither was mutagenic nor toxic.

Keywords: Diltiazem; Photodegradation; Photostability; Hydrolysis; HPLC analysis; Mutagenicity; Photoxicity

1. Introduction

Diltiazem, (2S,3S)-3-acetyloxy-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-2,3-dihydro-1,5-benzothiazepin-4(5H)-one, is a benzothiazepine calcium — channel blocker with peripherical and coronary vasodilator properties. It is widely used in the management of angina pectoris and hypertension [1,2]. Treatment with diltiazem is generally well tolerated; however, a variety of skin disorders have been associated with diltiazem therapy; these cutaneous adverse reactions include acne, rash, urticaria and dermatitis [2,3].

As regards the physico-chemical properties [4], diltiazem is considered a photolable compound and protection from the light has to be adopted for its storage [5,6]. In stability studies carried out in aqueous solutions the drug was found to give desacetyldiltiazem as the main degradation product [4,7–10]. This desacetyl derivative constitutes also an impurity from synthesis [4,11] and a major metabolite of the drug [4,11–13]. Exposure of aqueous solutions (pH 2 and 7) of diltiazem to UV radiations caused more degradation as compared to the same solutions protected from light [14]. These photochemical studies, however, appear to be quite preliminary, because only the remaining diltiazem was determined and the products from the hydrolytic and photolitic process were not distinguished.

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Thus, the evidences of adverse effects on the skin, although normally mild and transient, and the photoreactivity of diltiazem led us to further investigate the photostability of the drug in view of its potential phototoxic properties. In fact, the photoreactivity of the drugs can results in a photodegradation process of the drug (raw material and dosage forms) and can be also responsible for photo-induced in vivo interactions with endogenous compounds (phototoxicity).

Therefore, the present study was aimed to investigate the photodegradation of diltiazem, including isolation and identification of its main photoproduct(s). To this end, liquid chromatography (HPLC) was applied to monitor the photodegradation of diltiazem in aqueous solutions exposed to UVA–UVB radiations; the developed method was then applied to the quality control of commercial formulations of the drug. Moreover, in order to achieve preliminary information on the potential phototoxicity of diltiazem, in vitro photomutagenicty studies were performed using *Salmonella typhimurium* strains.

2. Experimental

2.1. Materials

Diltiazem was obtained from ICN Biochemicals Inc.(Germany) and diphenhydramine (the internal standard) was from Sigma (Italy). Methanol and acetonitrile for chromatographic analyses were of HPLC grade from Romil Analytical Sciences (UK). All other chemicals were from Carlo Erba Reagents (Italy). Water for solutions and buffers was obtained by the Milli-RX 20 System (Millipore).

Triethylammonium (TEA) buffer (pH 4; 0.01 M) solution was prepared by adding acetic acid to 0.01 M triethylamine aqueous solution up to the desired pH value. Phosphate buffer solutions (pH 4.0, 7.4 and 9.0; 0.01 M) were prepared according to standard methods.

2.2. Apparatus

The HPLC analyses were performed using a quaternary HP 1050 Ti series pump, equipped with a Rheodyne Model 7125 injector with a 20 μl sample loop. The eluates were monitored by a multiwavelength HP 1050 detector connected to a computer station (HP Chemstation, Vectra VT). For routine analyses the wavelength was set at 220, 240, 250 and 275 nm. The chromatographic separations were performed on a 10 μm RP-18 BondclonePhenomenex (300×3.9 mm i.d.) column, using mobile phases consisting of binary mixtures of acetonitrile-buffer solution whose composition was adjusted according to the specific application.

The NMR spectra were recorded on a Varian Gemini (200 MHz) NMR spectrometer, using TMS as the internal standard. The chemical shift is expressed in δ (ppm) and J in Hz with the following abbreviation: ar (aromatic). Mass spectra were recorded on a VG-Analytical (7070 E) mass spectrometer using an ionizing voltage of 70 eV.

Tests on the photochemical reactivity were carried out using a xenon arc source as artificial radiation system for simulating natural sunlight exposure. In particular, a 150 W xenon-arc lamp (solar simulator, model 68 805 Oriel Corporation, USA) was used, provided with a dicroic mirror (model 81 405) to block visible and IR radiations to minimize the sample heating. An air-mass filter (mod 81 090) was also used to simulate solar conditions. The output beam was directed downward by a 'beam turning assembly', which contain the dicroic mirror. The UV dose (J/cm^2) from the Xe-arc lamp was measured by a radiometer (Goldlux, mod 70 127, Oriel Corporation, USA).

For the photostability studies a UV-254 lamp (Helios Italquarz, Italy) was also used.

2.3. Alkaline hydrolysis of diltiazem

A series of 1.0 ml — volumes of a diltiazem solution (0.3 mg/ml) in water–methanol 9:1 (v/v) were treated with 0.5 ml of 5 mM sodium hydroxide solution; the alkaline solutions were then mantained at 25°C protected from the light. At prefixed times, the single samples were treated with diluted phosphoric acid up to obtain a pH 4 value and then subjected to HPLC analysis. TEA acetate buffer (pH 4.0; 0.01 M) — acetonitrile

72:28 (v/v) was used as mobile phase at a flow rate of 1.4 ml/min. UV detection at 220, 240 and 275 nm.

2.4. Photostability testing

Solutions of diltiazem (0.5 mg/ml) in phosphate buffer solutions (pH 7.4 and 9; 0.01 mM), and in TEA acetate buffer solution (pH 4.0; 0.01 M) into quartz cells were exposed to UVB–UVA radiations (xenon arc lamp). At selected times, the solutions were analyzed by HPLC to monitor the photodegradation of the drug. The solutions at pH 7.4 and 4.0 were directly injected into the chromatograph, while the pH 9 solution was treated with diluted phosphoric acid up to about pH 7 before the injection. The mobile phase consisted of TEA acetate buffer soluton (pH 4; 0.01 M) — acetonitrile 74:26 (v/v).

The effects of exposure to 254 nm radiations were also evaluated on diltiazem solution (0.2 and 0.4 mg/ml) in 0.1 M phosphate buffer solution (pH 7.4) using the same HPLC method.

2.5. Isolation of the photoproduct

A solution of diltiazem (0.2 mg/ml) in TEA acetate buffer solution (pH 4.0) was exposed to UVA–UVB radiations up to obtain essentially complete degradation of the drug. The irradiated solution was then saturated with sodium chloride and extracted with chloroform. The extracts were collected and the solvent was removed under vacuum; the residue was dissolved in methanol and analyzed by the describe HPLC. The residue was found to contain only the photoproduct and to be sufficiently omogenous for the subsequent NMR and mass spectrometry analyses.

2.5.1. Diltiazem

^{1}H-NMR ($CDCl_3$): δ 1.90 (3H, s, CH_3CO-), 2.90 (6H, s, $(CH_3)_2N$-), 3.25 (1H, m), 3.55 (1H, m), 3.84 (3H,s, CH_3O-), 4.45 (1H, m), 4.55 (1H, m), 5.03 (1H, d $J = 5.8$ Hz), 5.12 (1H, d, $J = 5.8$ Hz), 6.91 (2H, d, $J = 5.8$ Hz, Ar), 7.32 (2H, m, Ar), 7.36 (2H, d, $J = 5.8$ Hz, Ar),7.56 (1H, d, Ar), 7.69 (1H, d, Ar).

^{13}C-NMR ($CDCl_3$): δ 20.44, 43.27 ($(CH_3)_2N$-), 44.87, 54.08, 54.31, 55.37, 71.19, 113.90 ($2 \times$ C), 124.60, 126.08, 127.60, 128.35, 130.65 ($2 \times$ C), 132.01, 135.70, 144.28, 159.87, 168.13, 169.85.

2.5.2. Diltiazem S-oxide

^{1}H-NMR ($CDCl_3$): δ 1.87 (3H, s, CH_3CO-), 2.21 (6H, s, $(CH_3)_2N$-), 2.52 (2H, m), 3.62 (1H, m), 3.84 (3H, s, CH_3O-), 4.39 (1H, d, $J = 5.9$ Hz), 4.53 (1H, m), 5.26 (1H, d, $J = 5.9$ Hz), 6.95 (2H, d, $J = 5.86$ Hz, Ar), 7.42 (2H, d, $J = 5.86$ Hz, Ar), 7.48 (1H, m), 7.63 (2H, m), 7.98 (1H, m).

^{13}C-NMR ($CDCl_3$): δ 21.06, 30.35, 45.95 ($(CH_3)_2N$-), 47.71, 55.94, 57.48, 68.01, 77.42, 115.01 ($2 \times$ C), 123.96, 125.00, 129.34, 132.15 ($2 \times$ C), 132.98, 137.45, 138.66, 160.92, 165.96, 170.46.

Mass spectrum (EI): m/z (%): 430 (M^+; 0.1), 251 (1.11), 234 (1.26), 192 (1.83), 165 (1.62), 71 (68.64), 58 (100.00)

2.6. Calibration graph

Standard solutions of diltiazem (30–300 μg/ml) in TEA acetate buffer (pH 4.0; 0.01 M), containing a fixed concentration (100 μg/ml) of diphenhydramine (internal standard), were analyzed in triplicate by the described HPLC method using as mobile phase a mixture of TEA acetate buffer solution (pH 4.0; 0.01 M) – acetonitrile 74:26 (v/v). UV detection at 240 and 275 nm. The ratio of the drug peak area to internal standard peak area was plotted against the corresponding concentration to obtain the calibration graph.

2.7. Analysis of pharmaceutical formulations

2.7.1. Lyophilized product

The content (diltiazem and mannitol) was dissolved in water into a 5 ml calibration flask. Dilution 1–100 was then made with TEA acetate solution (pH 4; 0.01 M) containing the internal standard diphenidramine (100 μg/ml). Each sample was analyzed in triplicate by HPLC as described for calibration graph. The peak area ratios (analyte to internal standard) were determined and the drug content in the examined samples was calculated using the calibration graph.

2.7.2. Tablets

Five tablets were powdered and a 130 mg portion was treated with the pH 4 TEA acetate buffer into 20-ml volumetric flask. After sonication and dilution to volume, the resulting suspension was filtered and a 1-ml aliquot of the clear solution was diluted 1–10 with pH 4.0 TEA acetate solution to obtain a final sample solution containing 100 μg/ml of the internal standard. The usual procedure was then followed to determine the drug content in the sample.

2.8. Photostability of the reconstituted lyophilized preparation

The lyophilized content was dissolved in 5 ml of distilled water and the resulting diltiazem solution (10 mg/ml) was exposed to UVA–UVB radiation (solar simulator) into quartz cells. At selected times, aliquots were withdrawn and, after dilution 1–100 with TEA acetate solution containing the internal standard (100 μg/ml), were subjected to the described HPLC analysis. The peak area ratios of drug to internal standard were plotted against the time of exposure to UVA–UVB radiations.

2.9. Mutagenicity testing

The standardized plate incorporation assay was used with *S. typhimurium* TA 102 strain [15]. Diltiazem was dissolved in water and incorporated with the indicator strain (in the resulting phase) into an agar overlay. In the experiment in which activation was studied microsomal fraction obtained from Na–phenobarbital plus β-naphthoflavone induced rat liver was included in the assay. Toxicant was tested up to the toxicity or solubility limit. All plates were set up in duplicate; all experiments were reproduced at least twice. The spontaneous revertants frequencies for each plate ($\pm$ S.D.) were: TA 102, 325 ± 11.23

2.10. Photomutagenicity and phototoxicity testing

Exposure of bacteria to UV light was performed using an Osram Ultra-Vitalux sun lamp and the spectral radiation flux was calibrated before each experiment using an Osram Centra UV-meter, both as unfiltered light and as light filtered through the plastic cover or a 3 mm window pane. UVA and UVB measurements were recorded and doses calculated in mJ/cm^2, using the duration of exposure and dose rate.

Salmonella strains TA 102 was coplaned with diltiazem (10–300 μg/plate) in soft agar without S9 mix and irradiated with various doses of UV light. Plates were then processed as described before; 8-methoxypsoralene (8-MOP) was used as standard positive control [16–18].

3. Results and discussion

The preliminary objective of the work was to distinguish the products derived from the hydrolysis of diltiazem from those obtained by photochemical reactions. To this end, firstly the hydrolysis of diltiazem was studied on light protected solutions. To monitor hydrolytic and photochemical reactions selective HPLC methods were developed.

3.1. Hydrolysis of diltiazem

According to previously reported pH-stability profiles [7,8], an alkaline medium (pH 9) was chosen to carry out a rapid hydrolytic conversion of diltiazem to desacetyldiltiazem at ambient temperature. The reaction course was followed by a reversed phase HPLC method, which provided good resolution of the analytes (Fig. 1). According to previous reports [9,10] a single product was obtained, more hydrophilic than diltiazem; its UV spectrum (diode array detector), overimposable to that of the parent drug (Fig. 2), suggests that the drug chromophore was not modified. These data support a simple desacetylation reaction to give desacetyldiltiazem. Reactions involving the amide group in the seven-membered ring require more severe conditions [8] and should result in products with modified UV spectrum. Under the described hydrolysis conditions (pH 9; 25°C, 10% aqueous methanol) the conversion of diltiazem to its desacetyl derivative is rapid and follows a pseudo

first order process, according to the equation $A = A_0e^{-kt}$ ($n = 6$; $r^2 = 0.982$), where A is % remaining diltiazem peak area, k is kinetic constant and t is time (min). The following kinetic parameter were obtained: $k = 3.95 \times 10^{-2}$ min^{-1} and the half-life time $t_{0.5} = 17.6$ min.

3.2. Photodegradation studies

Diltiazem (0.5 mg/ml) in aqueous solutions at pH 4.0, 7.4 and 9.0 were exposed to UVA–UVB radiations from a solar simulator (xenon arc lamp) for a fixed time (15 h). The solutions were then analyzed by HPLC, using a modified mobile phase composition (lower % of organic modifier) to increase the retention and the resolution. A comparison of the resulting chromatograms (Fig. 3), shows that the pH value significantly affects the photodegradation process. In particular, at pH 4, where the hydrolysis rate is minimum [7,8], a single photoproduct is obtained that differs from both diltiazem and desacetyldiltiazem for the retention time (Rt = 21 min) and the UV spectrum (Fig. 2). At pH 7.4, the photodegradation proceeds similarly as at pH 4, with the formation of additional minor hydrophilic products. Differently, at pH 9.0 a serious degradation is observed, with the photoproduct (Rt = 21 min) well distinguished from the desacetylderivative (Rt = 11) and from other compounds. Therefore, under these conditions a complex degradation process occurs involving both hydrolytic and pho-

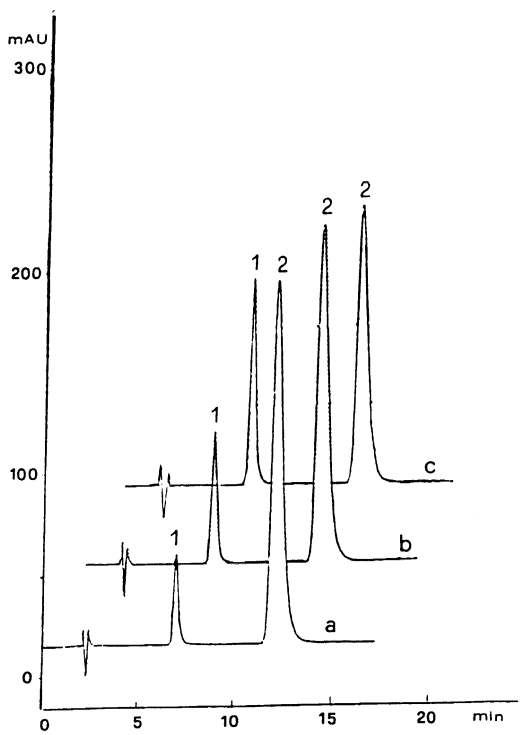

Fig. 1. Alkaline hydrolysis of diltiazem–HPLC chromatograms of diltiazem (0.3 mg/ml) in pH 9.0 aqueous solution after 4 min (a), 10 min (b) and 20 min (c) at 25°C. Peaks: (1) desacetyldiltiazem; (2) diltiazem. Chromatographic conditions: column: C-18 Bondclone (10 μm; 300 × 3.9 mm I.D.); mobile phase: TEA acetate buffer (pH 4; 0.01 M) — acetonitrile 72:28 (v/v) at 1.4 ml/min flow rate.

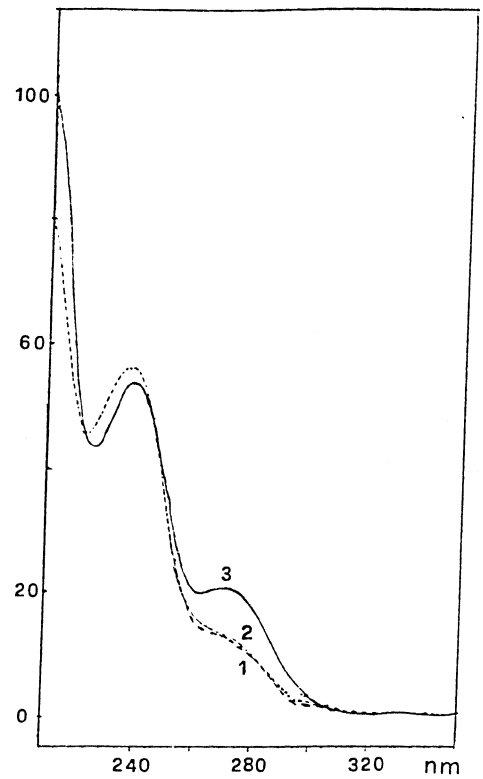

Fig. 2. UV spectra of diltiazem (1), desacetyldiltiazem (2) and diltiazem *S*-oxide (3) obtained by on-line diode array detector.

tochemical reactions. It should be emphasized that at physiological pH (pH 7.4) diltiazem undergoes significant photodegradation to a definite product, when exposed to UVA–UVB radiations. This makes of interest to investigate the photodegradation kinetic of the drug at pH 7.4 and also to isolate and to identify the resulting photoproduct.

Diltiazem solutions (0.2 mg/ml) at pH 7.4 were exposed to UVA–UVB radiations and the disappearance of the drug as well as the formation of the photoproduct was evaluated by HPLC (Fig. 4A). A linear relationship was obtained (Fig. 4B) according to the following equation: $\ln A = \ln A_0 - kt$, (apparent first-order kinetics; $r^2 = 1$),

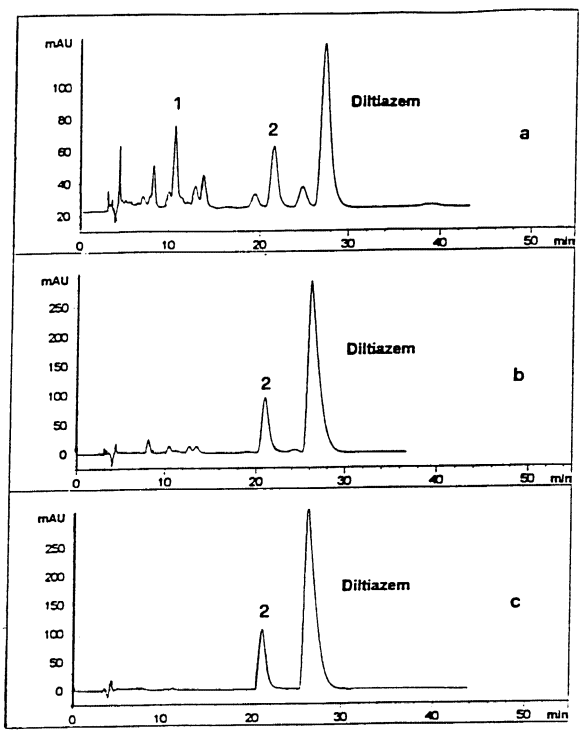

Fig. 3. HPLC chromatograms of diltiazem solutions (0.5 mg/ml) exposed to UVA–UVB radiation for 15 h; (a) solution in pH 9.0 phosphate buffer (0.01 M); (b) solution in pH 7.4 phosphate buffer (0.01 M); (c) solution in pH 4.0 TEA acetate buffer (0.01 M). Peaks: (1) desacetyldiltiazem; (2) diltiazem *S*-oxide. Chomatographic conditions: column as in Fig. 1; mobile phase: TEA acetate buffer (pH 4.0; 0.01 M) — acetonitrile 74:26 (v/v) at a flow rate of 1.0 ml/min.

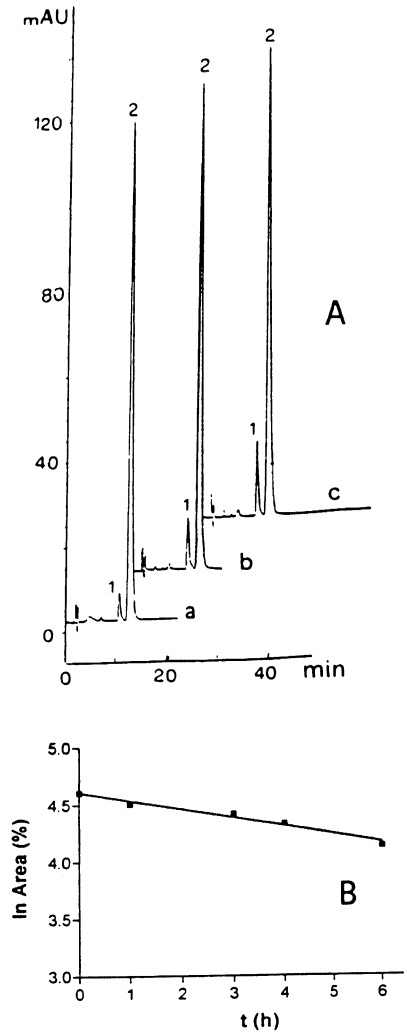

Fig. 4. Photodegradation of diltiazem at pH 7.4 and 25°C. (A) HPLC chromatograms of diltiazem solutions (0.2 mg/ml) exposed to UVA–UVB radiations for 1 h (a), 2 h (b) and 3 h (c). Peaks: (1) diltiazem-*S*-oxide and (2) diltiazem. Chromatographic condition as in Fig. 1. (B) Photodegradation profile of diltiazem (pH 7.4) under exposure to UVA–UVB radiations (25°C).

where A is the remaining peak area, k is slope and t is time (h). The following data were obtained: slope (rate constant) $= -0.074$/h and $t_{0.5} = 9.36$ h.

The photodegradation was also evaluated under exposure to UV 254 nm radiations at two concentration levels (0.2 and 0.4 mg/ml), with

absorbance values >2 at this wavelength. As expected, the photodegradation process was found to be slower at higher concentration, likely because the part of the drug more inside the volume is protected from light and does not partecipate in the photoreaction [19].

3.3. Identification of the photoproduct

Diluted diltiazem solutions at pH 4.0 were subjected to phodegradation. Under these conditions only the photoproduct at Rt = 21 min (Fig. 3) was obtained and extracted by liquid–liquid extraction with chloroform. The isolated compound was identified by NMR and mass spectra as diltiazem *S*-oxide (III) (Fig. 5). In particular, the proton NMR spectrum shows the upfield shift of the proton at C2 (δ 4.39) compared to that of diltiazem (δ 5.03) [11] and the carbon NMR spectrum shows the shift of a -CH- to 77.42 ppm (photoproduct) from 54.08 (diltiazem) due to the magnetic anisotropy of the newly formed S→O bond. This structural assignment is supported by the mass spectrum, which shows the molecular ion at m/z 430, according to addition of an oxygen atom to diltiazem (MW 414). The formation of the *S*-oxide (and not *N*-oxide) is confirmed by

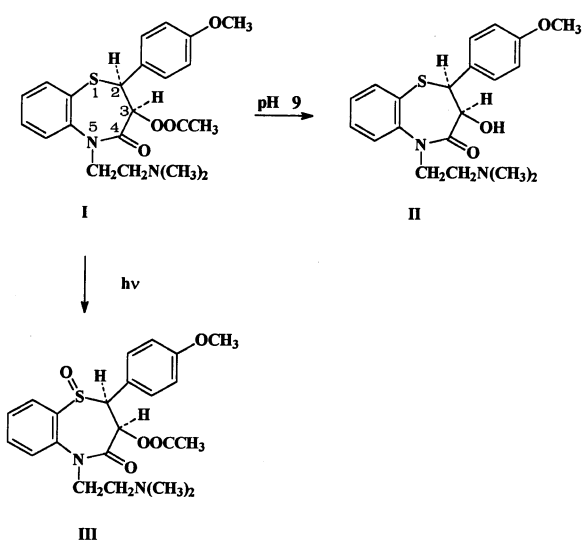

Fig. 5. Scheme of degradation of diltiazem (I) to desacetyldiltiazem (II) and diltiazem *S*-oxide (III).

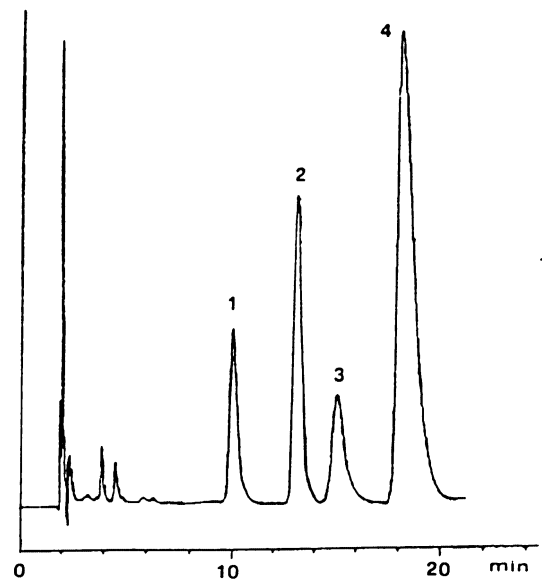

Fig. 6. Representative HPLC separation of desacetyldiltiazem (1), internal standard (diphenhydramine) (2), diltiazem *S*-oxide (3) and diltiazem (4). Column as in Fig. 1; mobile phase: TEA acetate buffer (pH 4.0; 0.01 M) — acetonitrile 74–26 (v/v) at a flow rate of 1.4 ml/min.

the presence of the fragments at m/z 58 ($H_2C{=}N^+(CH_3)_2$) and 71 due to the intact dimethylaminoethyl chain [4] and by the absence of the fragment (M^+-$(CH_3)_2$N-OH) typical of diltiazem *N*-oxide derivatives [11].

3.4. Analysis of pharmaceutical formulations

The HPLC method developed for the photodegradation studies was also applied, with minor modification in the mobile phase composition, to the analysis of commercial formulations (tabled and lyophilized). Quantitative analyses were carried out using difenidramine as internal standard, being separated from diltiazem and its photo- and hydrolytic degradation products (Fig. 6). For quantitative applications a linear relationship was found between the peak height ratio (drug to internal standard) (Y) and the drug concentration C (μg/ml): $Y = (6.970 \pm 0.041)C - (0.040 \pm 0.007)$ ($n = 5$; $r = 0.9997$).

The sample preparation involved simple dissolution and filtration steps. The HPLC analysis

Table 1
Photomutagenicity of diltiazem on TA102 strain of *S. typhimurium*

Dose (μg/plate)	Revertant/plate (UVA/UVB in mJ/cm^2 per min)				
	0	212/0.00	254.4/0.00	339.2/0.00	424/0.00
DMSO	329 ± 9.19	365 ± 23.33	403 ± 21.21	449 ± 23.33	416 ± 11.31
10	354 ± 14.85	376 ± 37.77	406 ± 19.80	362 ± 38.89	407 ± 12.02
30	338 ± 4.24	352 ± 8.49	413 ± 33.23	468 ± 19.08	366 ± 34.65
100	362 ± 6.36	474 ± 14.85	389 ± 3.54	439 ± 24.04	436 ± 5.66
300	379 ± 7.78	463 ± 12.73	334 ± 10.09	379 ± 43.13	405 ± 18.38

provided the following recovery values: 101.12% (R.S.D.% = 0.373) for the lyophilized product, and 101.86% (R.S.D.% = 0.306) for tablets.

On account of the recommendations to avoid light exposure of diltiazem preparations, the photostability of the reconstituted solution from the lyophilized product was evaluated. An aliquot of the resulting diltiazem solution (10 mg/ml) in quartz cells was exposed to UVA–UVB radiations for 28 h, and then analyzed by HPLC. Photodegradation was observed only in a limited extent (5.6%). Therefore, it is reasonable to draw that reconstituted high concentration diltiazem solutions, stored in glass container able to eliminate the UVB light component, are adequately photostable over the usual assumption period.

3.5. Photomutagenicity and phototoxicity

Phototoxicity testing and photomutagenicity test systems have been used to test the toxic reactions evoked by exposure of diltiazem to UV radiations. As shown in Table 1, diltiazem (tested up to 300 μg/plate) in absence of light neither was mutagenic nor toxic for *S. typhimurium*. UV exposure alone (with a reduced UVB component by a 3-mm glass window pane) had a negligible effect on the rate of gene mutations at the dose employed. The product of the light–diltiazem reaction did not induce mutations in the excision-repair proficient strain TA102 (Table 1). Toxic effects were not evident either at the highest doses of diltiazem. On the other hand 8-MOP (0.1 μg/μl in DMSO), the suitable test compound for esthablishing bacteria photomutagenicity assay, showed clear UV dose-related mutagenicity ($P < 0.01$) in TA102 strain (data not shown).

4. Conclusions

Diltiazem in diluted aqueous solutions, exposed to UVA–UVB radiations, was found to be photolable, giving diltiazem-*S*-oxide as the main photodegradation product. This compound constitutes essentially the only photoderivative at pH 4.0 and 7.4, whereas at pH 9.0 more complex degradation occurs involving both hydrolytic and photochemical reactions. These processes were monitored by stability-indicating HPLC analysis that allowed to confirm the substantial photostability of a reconstituted high concentration diltiazem commercial solution. In a preliminary photomutagenicity testing toxic effects by diltiazem were not observed.

Acknowledgements

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