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Photodegradation studies on Atenolol by liquid chromatography

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Abstract

The photostability of the β-blocker drug Atenolol was evaluated at pH 9, 7.4 and 4.0. The drug was exposed to UVA–UVB radiations and the photoproducts were detected by reversed phase LC methods. The photodegradation was found to increase with the pH value decreasing. The major photodegradation product at pH 7.4 was identified as 2-(4-hydroxyphenyl)acetamide. The LC method developed for routine analyses (column: C-18 Alltima; mobile phase: TEA acetate (pH 4; 0.01 M)–acetonitrile 96:4) was found to be suitable for the stability — indicating determination of Atenolol in pharmaceutical dosage forms. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Atenolol; Photostability; Liquid chromatography; Quality control

1. Introduction

Drug photostability constitutes an important current subject of investigation because the photodegradation process can result in a loss of the potency of the drug and also in adverse effects due to the formation of minor toxic degradation products [1–5]. As a consequence, various pharmacopoeias prescribe light protection for a number of drugs and adjuvants during storage. Knowledge of the photochemical and photophysical properties of drugs is essential to ensure adequate product quality and also for predicting drug phototoxicity. To this end, specific guidelines for the photostability testing on drugs have been proposed by the International Conference on Harmonization (ICH) [1,6].

Some classes of drugs have been investigated thoroughly for their photostability and phototoxicity [7–11]; however, for other drugs only limited information has been reported and specific, confirmatory studies have not been performed. Thus, the lack of detailed studies on the photostability of β-blocker drugs focused our attention on this class of drugs; in particular, the present communication deals with the photostability of Atenolol under UVB (290–320 nm) and UVA (320–400 nm) radiations. The photodecomposition of the drug in aqueous solutions was monitored by a selective liquid chromatographic (LC) method, which proved to be suitable for a reliable quality control of commercial formulations (tablets).

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2. Experimental

2.1. Materials

Atenolol (Sigma-Aldrich, Milan, Italy) and 3,4-dimetoxyaniline (Fluka Chemika, Buchs, Switzerland) were used as received. Methanol and acetonitrile of LC grade were from Promochem (Germany); all the other chemicals were from Carlo Erba Reagents (Milan, Italy). Water double distilled and filtered through a 0.45 μm filter was used to prepare all solutions and buffers. Phosphate buffer solutions (pH 7.4; 0.01 M), ammonium acetate buffer (pH 7.0; 0.01 M) and triethylammonium (TEA) acetate buffer (pH 4.0; 0.01 M) were prepared according to standard methods.

Solid phase extraction (SPE) was performed on BondElut (Varian) Cartridges (500 mg of C-18 sorbent) conditioned by rinsing with 3 ml of methanol and then 3 ml of the appropriate buffer.

2.2. Apparatus

The LC 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, 270 and 335 nm. The chromatographic separations were performed on a RP-18 Alltima, Altech (150 × 4.6 mm) column and the mobile phase composition was adjusted according to the reported applications. The NMR spectra were recorded on a Varian 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 abbreviations: ar = aromatic, br = broad. GC–MS analyses were performed on a HP 5890 series II gas chromatograph with a mass selective detector HP 5971.

2.3. Photostability studies

For the UV radiation exposure testing a 150 W xenon arc lamp (solar simulator, model 68805 Oriel Corp., USA) was used, provided with a dicroic mirror (model 81405) to block visible and IR radiation to minimize the sample heating. An air-mass filter 1.5 (model 81090) was also used to simulate solar conditions. The output beam was directed downward by a 'beam turning assembly', which contains the dicroic mirror.

The UV dose (J/cm^2) from the Xe arc lamp was measured by a radiometer (Godilux, model 70127, Oriel Corp.).

2.3.1. Forced degradation

Atenolol solutions (0.2 mg/ml) prepared in pH 9.0, 7.4 and 4.0 buffers into 1 cm (3 ml) quartz cells were subjected to UVA and UVB radiations (xenon arc lamp) for 17 h, equivalent to doses of 184.00 J/cm^2 (UVA) and 29.6 J/cm^2 (UVB). The irradiated solutions were then analyzed by LC on a C-18 stationary phase using as mobile phase ammonium acetate (pH 7; 0.01 M) (A)–methanol (B), under isocratic (90:10 v/v) or gradient conditions $t = 0$ A = 95%; $t = 5$ min, A = 95%; $t = 20$ min, A = 80%. The flow rate was 0.8 ml/min.

The rate of photodegradation was evaluated by exposure of solutions of Atenolol (0.2 mg/ml) in TEA acetate buffer (pH 4.0; 0.01 M) and phosphate buffer (pH 7.4; 0.01 M) to UVA and UVB radiations at 25 ± 1°C. Samples were withdrawn at appropriate intervals for LC analysis using a mobile phase consisting of the binary mixture ammonium acetate (pH 7; 0.01 M) (A)–acetonitrile (B) whose composition was varied according the following gradient: $t = 0$, A = 95%; $t = 20$ min, A = 80%.

2.3.2. Isolation of the photoproducts

2.3.2.1. pH 7.4. Atenolol solution (2 mg/ml) in pH 7.4 phosphate buffer was subjected to UVA–UVB radiations for 15 h and then 3 ml of the solution were transferred on a conditioned C-18 SPE column. Washing with water–methanol 90:10 (v/v) provided the selective elution of the photoproduct with $t_r = 5.36$ (Fig. 1(a)). Replicate SPE process were performed, the eluates were collected, saturated with sodium chloride and extracted with ethyl ether. The solvent was removed under vacuum and the solid residue, homoge-

neous by LC analysis, was subjected to NMR and mass spectrometry analyses.

^{1}H NMR (DMSO-d_6): δ 3.22 (2H, s, CH_2CO–), 6.67 (2H, d, $J = 8.38$, ar.), 6.79 (1H, s br., NH_2, D_2O exchang.) 7.04 (2H, d, $J = 8.52$), 7.34 (1H, s br., NH_2), 9.22 (1H, s br., –OH, D_2O exchang.). ^{13}C (DMSO-d_6): 51.19; 12.68 ($\times$ 2); 136.37; 139.64 ($\times$ 2); 165.56; 182.48. Mass spectroscopy (70 eV): 151 (M^+; 25), 107 ($M^+ - CONH_2$; 100).

2.3.2.2. pH 4.0. Atenol solution (2 mg/ml) in pH 4.0 TEA acetate buffer was irradiated for 15 h and then 3 ml aliquots were subjected to the SPE procedure. Using mixtures methanol–water the hydrophilic photoproducts and the residual Atenolol were first removed; and then using methanol the lipophilic photoproduct ($t_r = 24.5$ in Fig. 1(b)) was recovered and the methanolic solution was analyzed by GC–MS.

GC–MS: 248 (M^+; 11), 234 ($M^+ - CH_2$; 100)

2.4. Analysis of Atenolol formulations

2.4.1. Calibration graph

Standard solutions of Atenolol (0.1–0.8 mg/ml) in water, containing the internal standard

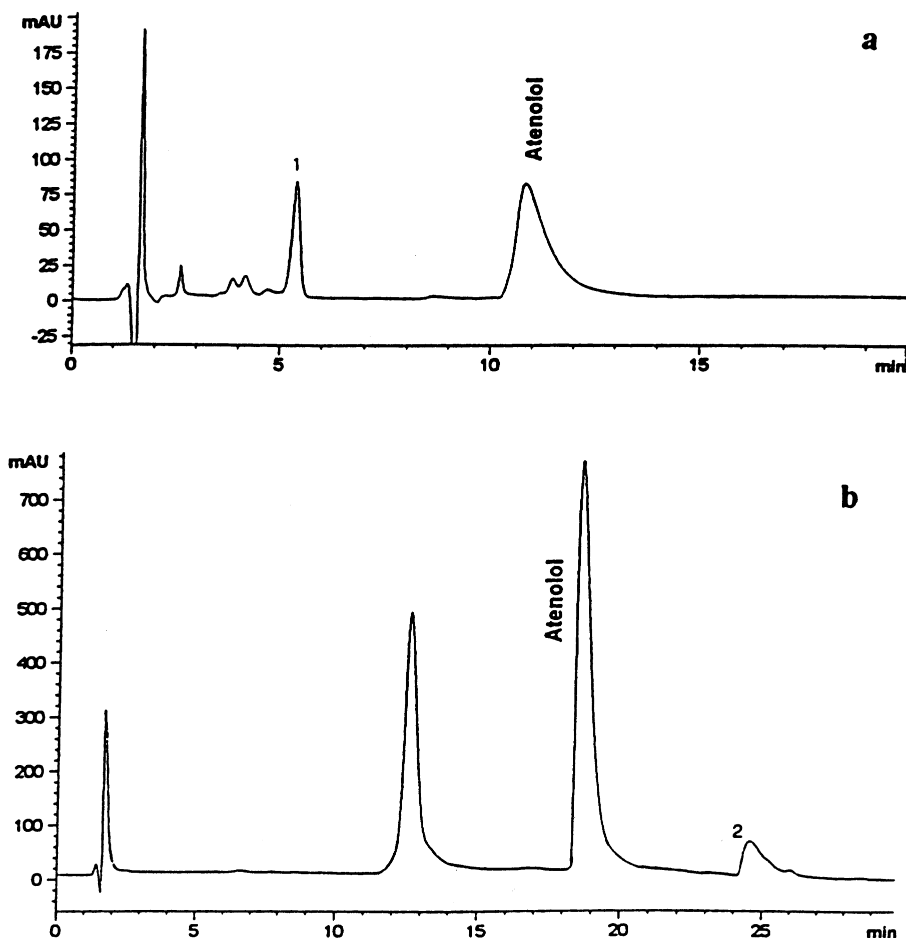

Fig. 1. LC chromatograms of Atenolol solutions (pH 7.4) exposed to UVA–UVB radiations (xenon arc lamp) for 17 h. (a) Atenolol solution (0.2 mg/ml); column: C-18 Alltima (150 × 4.6 mm); mobile phase: ammonium acetate solution (pH 7; 0.01 M)–methanol 90:10 (v/v) at a flow rate of 0.8 ml/min. (b) Atenolol solution (1 mg/ml); column as (a) using gradient elution with ammonium acetate (pH 7, 0.01 M) (A)–methanol (B): $t = 0$, A = 95%; $t = 5$, A = 95%; $t = 20$, A = 80%.

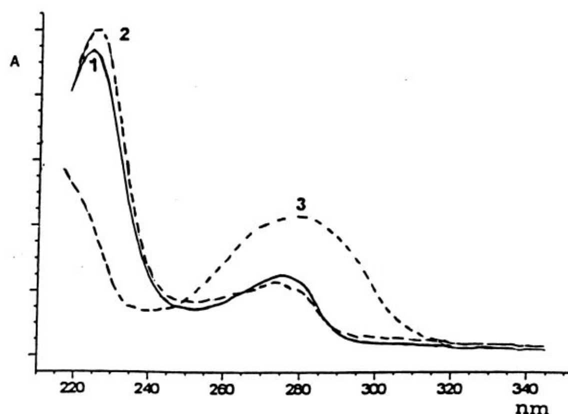

Fig. 2. UV spectra (diode array detector) of the compounds relative to the chromatographic peaks of Fig. 1(b). (1) Atenolol ($t_r = 18.7$), (2) photoproduct at $t_r = 12.5$, and (3) photoproduct at $t_r = 24.5$.

3,4-dimetoxy aniline (0.050 mg/ml), were injected in triplicate into the chromatograph. The chromatographic separation was performed using TEA acetate (pH 4; 0.01 M)–acetonitrile 96:4 (v/v) at a flow rate of 0.8 ml/min. The peak area ratios (analyte to internal standard) were plotted against the corresponding analyte concentration.

2.4.2. Assay procedure

A sample (40 mg) of the powdered tablets was introduced into a 20 ml volumetric flask; 10 ml of water–methanol 90:10 (v/v) and 2 ml of the internal standard solution (0.5 mg /ml in pH 4.0 TEA acetate) were added, diluting to the correct volume with water. The resulting suspension was filtered through a 0.45 ml filter (Millipore) and the clear solution obtained was analyzed by LC, as described in Section 2.4.1.

3. Results and discussion

Acceleration tests on the photochemical reactivity of Atenolol were carried out using a xenon arc source as an artificial radiation system for simulating natural sunlight exposure. These controlled conditions constitute a viable option according to the ICH guidelines [1]. The UV spectrum of Atenolol exhibits a maximum at 275 nm and, therefore, the UVB component of the sunlight can be considered the main responsible for the photochemical reactivity of the drug.

The drug solutions were analyzed by LC under reversed-phase conditions. Ammonium acetate (pH 7.0) buffer (low buffer capacity), used only for monitoring the Atenolol photodegradation, was chosen to prevent additional hydrolytic degradation in the mobile phase and in view of eventual LC–MS analyses, where volatile buffer are required. Routine LC analyses of Atenolol in dosage forms were performed using a mobile phase containing a pH 4.0 phosphate buffer solution.

3.1. Photodegradation studies

Preliminary tests were performed under forced conditions in order to induce significant photodecomposition useful to develop selective LC methods essential for these studies. Therefore, Atenolol solutions (0.2 mg/ml) in pH 9, 7.4, and 4 buffers were exposed to UVA and UVB radiations for 17 h. The obtained results can be summarized as follows: (a) Atenolol appears to be relatively stable at pH 9, whereas its photodegradation increases as the pH value decreases; (b) At pH 7.4, the principal photoproduct ($t_r = 5.36$) is more hydrophilic than Atenolol ($t_r = 10.85$) (Fig. 1(a)), while a more lipophilic product is obtained only in minor extent ($t_r = 24.5$) (Fig. 1(b)). This lipophilic photoproduct is obtained in higher yield at pH 4. Using a diode array detector (DAD), the UV spectra of the observed photoproducts were compared with that of the parent drug (Fig. 2). As shown, the hydrophilic product exhibits an UV spectrum similar to that of Atenolol, whereas the more lipophilic one displays a significantly modified chromophore. Moreover, the hydrophobic products obtained under pH 7.4 and pH 4 conditions, were confirmed to be identical on the basis of their superimposable UV spectra.

Photodegradation of pH 7.4 solution (physiological pH) was considered of interest, in view of potential phototoxic effects and, therefore, the work was directed to isolate and characterize the two principal products obtained under these conditions. For preparative purposes, the photode-

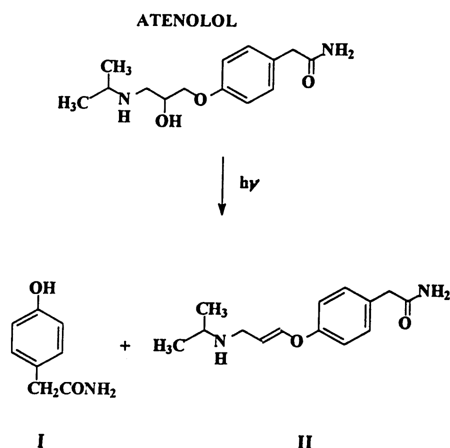

Fig. 3. Scheme of the photodegradation of Atenolol in pH 7.4 solution.

composition was carried out at pH 7.4 to isolate the hydrophilic product (I), while the UVA–UVB irradiation was carried out at pH 4 to improve the yield of the hydrophobic compound (II). The irradiated solutions were then subjected to a solid-phase extraction (SPE) to isolate the photoproducts. The SPE procedure to obtain the hydrophilic photoproduct (I) involved a single step elution with a mixture water–methanol having high water content (90%) in order to separate the product(I) from the residual Atenolol. Differently, to isolate the hydrophobic product (II), a first washing step with mixtures of methanol–water allowed the more hydrophilic compounds and Atenolol to be selectively eliminated. The recovery of photoproduct (II) was then obtained with 100% methanol.

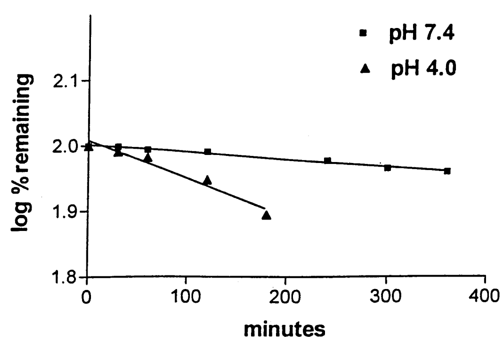

Fig. 4. Photodegradation of Atenolol in pH 7.4 and pH 4 solutions (0.2 mg/ml)under exposure to UVA–UVB radiations at 25°C.

The main derivative (I) was characterized as 2-(4-hydroxyphenyl)acetamide on the basis of its NMR and mass spectra (experimental section), as a result of the photolysis of the ethereal bond. In particular, the proton NMR spectrum shows the exchangeable hydroxyphenyl proton as a broad singlet at δ 9.22; the presence of the acetamide group is clearly visible as a signal at δ 3.22 for $-CH_2CO-$ and two signals, broad singlets at δ 6.79 and δ 7.34, exchangeable with D_2O, for the amide group.

GC–MS analysis suggested that dehydration of Atenolol was also involved in the photochemical reaction to give the product (II) at trace level at pH 7.4. Therefore, the photodegradation process of Atenolol in pH 7.4 solution can be summarized as shown in Fig. 3. The degradation mechanism at pH 4.0 appears to be the same up to 3 h, but for longer light exposure additional hydrophilic photoproducts are produced.

3.2. Degradation kinetic

According to recommendations for preliminary photodegradation studies [12], the rate of the Atenolol photodegradation was evaluated in low concentration aqueous solutions, so that the drug does not absorb all the light available and the reaction rate is limited by the drug concentration. Solutions of the drug at pH 7.4 and pH 4.0 under exposure to UVA–UVB radiations were monitored by LC and the resulting degradation profiles are illustrated in Fig. 4. As shown, linear plots of log of remaining drug concentration against time (min) were obtained at pH 7.4 (correlation coefficient (r) = 0.982) and pH 4.0 (r = 0.957) according to apparent first-order kinetics. From the slopes of the plots the following kinetic parameters were estimated: the rate constants k: 2.55×10^{-4}/min (pH 7.4) and 1.73×10^{-3}/min (pH 4); the half-life time $t_{0.5}$: 45.2 h (pH 7.4) and 6.87 h (pH 4); the time of the decomposition of 10% of the drug $t_{0.1}$: 6.85 h (pH 7.4) and 1.01 h (pH 4). Although these kinetic parameters depend on the experimental conditions (radiation source and sample irradiation geometry, ion and solvent ef-

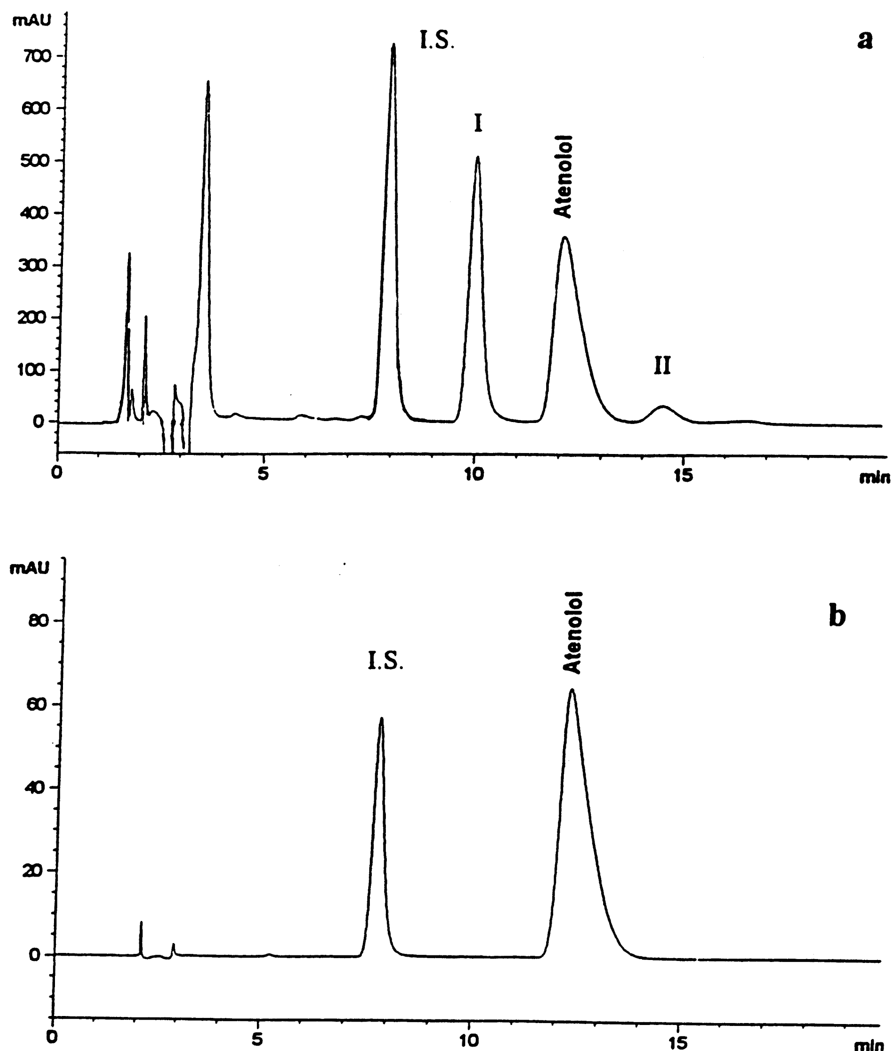

Fig. 5. Representative LC chromatograms obtained from: (a) an Atenolol solution (0.2 mg/ml) in pH 7.4 buffer solution (0.01 M) exposed to UVA–UVB radiation for 15 h; (b) a sample solution from a commercial Atenolol dosage form. Chromatographic conditions: column as in Fig. 1; mobile phase: TEA acetate (pH 4; 0.01 M)–acetonitrile 96:4 (v/v) at a flow rate of 0.8 ml/min.

fects), their relative values are of practical utility, suggesting the need for appropriate light protection of Atenolol for its handling and storage, particularly in acidic medium.

3.3. Analysis of commercial formulations

On account of the photoreactivity of Atenolol, a reliable quality control of the drug formulations should claim for selective, stability-indicating methods. Therefore, a selective LC method able to discriminate the drug from its photodegradation products was developed and applied to the analysis of commercial dosage forms. A representative LC separation of the analytes and the internal standard (3,4-dimethoxyaniline) is illustrated in Fig. 5(a). Under these chromatographic conditions the resolution of all the photodegradation products was achieved in a short analysis time with good peak symmetry.

For quantitative applications a linear relationship was obtained between the peak area ratio (analyte to internal standard) (y) and the drug concentration (x; μg/ml):

$$y = (0.00552 \pm 0.00005)x + (0.00397 \pm 0.02827) \quad (n = 8;\ r = 0.998).$$

The intra-day precision of the method, expressed as RSD (%) from replicate ($n = 5$) analyses of the same standard solution was satisfactory (RSD% = 1.015 at 100 μg/ml level and 0.750 at 500 μg/ml level). The inter-day precision ($n = 10$) was: RSD%: 1.54 and 1.35, respectively, at the indicated levels.

A commercially-available dosage form (tablets) of Atenolol (100 mg/tablet) was analyzed. Other formulation ingredients were: magnesium carbonate, magnesium stearate, starch, gelatin and sodium lauryl sulfate. The sample preparation was simple involving conventional dissolution and filtration procedures; the LC assay confirmed the drug content in close agreement with the claimed content (% found: 101.73; RSD%: 1.04). A typical chromatogram obtained for a commercial sample is shown in Fig. 5(b); as can be seen, no significant amounts of photoproducts have been detected. The accuracy of the method was evaluated by recovery studies analyzing samples spiked with known quantities of the drugs and essential quantitative recoveries were obtained.

In summary, Atenolol was found to be photoreactive when exposed to UVA–UVB radiations and, therefore, adequate light protection should be adopted for its storage and handling. The limited extent of the photodegradation of the drug at physiological pH and its UV absorption restricted to the UVB region should exclude possible phototoxic effects. Future in vitro phototoxic studies for Atenolol and other β-blocker drugs are planned.

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