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Different photodegradation behavior of barnidipine under natural and forced irradiation

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ABSTRACT

An in depth study on photodegradation of barnidipine, a new 1,4-dihydropyridine antihypertensive drug, was performed by exposing the drug to natural and stressing light irradiation. A different degradation process and distribution of photoproducts under different light sources and irradiation power were demonstrated. Degradation kinetics, reactive excited states involved, transient species and photoproducts generated were investigated. Exposure of barnidipine to forced irradiation caused a complex degradation pathway. Kinetics of this process was studied by HPLC and spectrophotometry, demonstrating the formation of several by-products. In contrast, the drug, under direct or indirect sunlight, underwent oxidation generating the pyridine derivative as the main photodegradation product, according to most of the drug congeners. HPLC and derivative spectrophotometric methods were defined for the simultaneous assay of the drug and its pyridinic by-product, useful as a routine control method of the drug formulations.

1. Introduction

Barnidipine (BAR) [3-(3R)-1-benzylpyrrolidin-3-yl-5-methyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate] is one of a new generation of 1,4-dihydropyridine calcium-channel blockers [1], widely used against hypertension and angina [2–6].

The 1,4-dihydropyridine antihypertensives are well-known photosensitive drugs. The most common photodegradation pathway is the oxidation to the pyridine derivatives, lacking any therapeutic effect [7–12]. This degradation mechanism has recently attracted considerable attention because it has been demonstrated that metabolism of those drugs involves an analogous cytochrome P-450 catalyzed oxidation in the liver [13]. Some 1,4-dihydropyridine drugs undergo a more complex degradation with the formation of secondary photoproducts [14–16].

In the literature there are no studies about photodegradation kinetics of BAR. In a recent publication, the influence of its chemical substituents on photosensitivity has been discussed [7]. Details on the photodegradation mechanism still remain unclear as well as the sequential degradation steps that may take place. Usually, the prediction of the photochemical behavior of a molecule is a very complex problem [17], because the photodegradation mechanism depends not only on the structure but also on experimental conditions.

In the present work, the photodegradation behavior of BAR under stressing light and natural light was evaluated and the relative degradation pathways and kinetics under these irradiation conditions were compared. Photodegradation was conducted in accordance with the recommendations of the "Guide for the Photostability Testing of New Drug Substances and Products" developed by the International Conference on Harmonization (ICH) [18]. This guideline addresses the evaluation of stability data that should be submitted in registration applications for new drugs and associated drug products and provides recommendations on establishing retest periods and shelf lives. The guideline refers light testing as an integral part of the global evaluation for stability data to be performed. Testing should demonstrate that light exposure does not result in unacceptable changes and be performed on pure drug and drug products. Photostability tests should evaluate the overall photosensitivity of a drug for method development purposes and degradation pathway elucidation and then provide the information necessary for handling, packaging, and labelling. Detailed articles on the ICH guideline and its application have been published [19–21].

In order to avoid different testing procedures, the guideline gives an accurate definition of the light sources to be adopted. In this work, the stressing tests were conducted by means of a cabinet

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equipped with a Xenon lamp, in accordance with one of the light sources reported in the guide. Degradation in natural conditions was conducted under daylight and direct sunlight.

BAR photodegradation was monitored by HPLC and zero-crossing derivative spectrophotometry. Adoption of the spectrophotometric analysis was justified by the easy recording of the spectra and the rapid interpretation of the data, which makes this technique very attractive for routine uses. Zero-crossing is a powerful technique for the quantitative assay of the components of a mixture, particularly effective when a wide peaks overlapping is present in the corresponding zero-order spectrum [22–27].

HPLC analysis allowed to evaluate in detail the formation of all the photoproducts and hypothesize the sequential degradation steps that may take place during the light exposure under natural and forced conditions. The study of the photochemical properties of BAR were useful to understand the transient species produced after light absorption. An analytical method based on zero-crossing derivative spectrophotometry was also developed to be used as a routine quality control for BAR and its pharmaceutical formulations.

2. Materials and methods

2.1. Chemicals

BAR was obtained by extraction from the specialty Libradin® 20.0 mg (Sigma-Tau S.p.A., Italy). The content of five capsules was pulverized in a mortar and the equivalent to one capsule was suspended in ethanol in a 20 ml volumetric flask. The suspension was sonicated for 10 min and then filtered through a PTFE 0.45 μm membrane to obtain a stock solution of 1 mg/ml.

The pyridine by-product (BOX) was obtained by exposing under daylight the BAR stock solution in a 20 ml quartz volumetric flask for 5 h. Purity and identity of the photoproduct were confirmed by HPLC-DAD analysis.

Ethanol and acetonitrile were of instrumental purity grade (J.T. Baker, Holland). PTFE 0.45 μm membrane filters were purchased from Supelco (Milan, Italy). All other reagents were of the highest purity commercially available.

2.2. Instrumentation

Zero-to-fourth order derivative spectra were registered on the λ range of 200–450 nm in a 10 mm quartz cell, by means of a Perkin-Elmer Lambda 40P spectrophotometer at the following conditions: scan rate 1 nm/s; time response 1 s; spectral band 1 nm. The software UV Winlab 2.79.01 (Perkin-Elmer) was used for spectral acquisition and elaboration.

HPLC analysis was carried out by using a HP 1100 pump fitted with a diode array detector G1315B (Agilent Technologies) and a Rheodyne 7725 manual injector. The LC column was a C18 Gemini (Phenomenex), 250 × 4.6 mm × 5 μm. The mobile phase consisted of acetonitrile–phosphate buffer (pH 7) (75:25, v/v) at room temperature. The mobile phase was filtered through a 0.45 μm nylon membrane under vacuum. The mobile phase was pumped isocratically at a flow rate of 1 ml/min during analysis. The injection volume was 5 μl.

Gas-chromatographic (GC–MS) analysis was performed by using a gas chromatograph Agilent 6890N with a Mass Selective Detector Agilent 5973. The GC conditions were as follows: column, HP capillary (30 m × 0.25 mm I.D.), 250 nm film thickness; injection port temperature, 250 °C, carrier gas, helium; flow-rate 0.3–0.6 ml/min; column temperature, programmed from 60 to 280 °C at 13 °C/min, initial time 3 min, final time 54 min.

Forced photodegradation was conducted in a light cabinet Suntest CPS+ (Heraeus, Milan, Italy), equipped with a Xenon lamp. The apparatus was fitted up with an electronic device for irradiation and temperature measuring and controlling inside the box. The system was able to select spectral regions by interposition of filters. In the present study, samples were irradiated in a λ range between 300 and 800 nm, by means of a glass filter, according to the ID65 standard of ICH rules.

The steady state fluorescence experiments were carried out on a spectrofluorometer system (Photon Technology International, Birmingham, NJ), equipped with a monochromator in the wavelength range of 200–900 nm. The solutions were placed in 10 mm quartz cells with a septum cap.

The Laser Flash Photolysis (LFP) experiments were conducted by using a Q-swiched Nd:YAG (neodymium-doped yttrium aluminium garnet) laser coupled to a turn-key mLFP-miniaturized equipment from Luzchem (Ottawa, ON, Canada). Samples were prepared in acetonitrile and purged with nitrogen for 10 min in a 10 mm fluorescence quartz cell, then sealed with a septum before irradiation at 355 nm. The single pulse was of about 17 ns and the energy was below 100 mJ/pulse.

2.3. Standard solutions

Standard solutions of the pure products BAR and BOX in ethanol were prepared and used to set up the spectrophotometric calibration curves. BAR concentration was within the range 5.0–50.0 μg/ml and BOX in the range 0.2–50.0 μg/ml. A second set of standard mixtures within the above reported concentrations were prepared, with BOX percentage value between 1% and 50%. These samples were used to validate the elaborated methods.

For HPLC analysis, a series of standard solutions in ethanol with both BAR and BOX within the range 20.0–400.0 μg/ml were prepared to set up the calibration curves.

2.4. Sample solutions

The content of five capsules was pulverized and the equivalent to one capsule was suspended in ethanol in a 20.0 ml volumetric flask. The suspension was sonicated for 10 min and then filtered through a PTFE 0.45 μm membrane. Samples for UV analysis were obtained after serial dilution 1:100 of this filtrate with ethanol and analyzed. For HPLC assay, samples were obtained by diluting 1 ml of the filtrate to 10.0 ml with ethanol.

2.5. Photodegradation experiments

To minimize BAR photodegradation, all laboratory experiments were carried out in a dark room under the illumination of a red lamp (60 W), kept at a distance of about 2 m.

The stressing tests were performed on a series of BAR solutions in quartz cells perfectly stoppered and irradiated in the light cabinet, according to the ID65 standard of the ICH rules. The irradiation power was sequentially fixed at 250, 350, 450, 550 and 650 W/m^2 (corresponding to 15, 21, 27, 33 and 39 kJ/m^2 min of irradiance, respectively) at a constant temperature of 25 °C. Samples with BAR concentration of 20.0 and 200.0 μg/ml were assayed by spectrophotometry and HPLC, respectively, without further treatment. Both the techniques were performed just after preparation (t = 0 min) and every minute until 100 min.

These forced conditions were also applied to test the stability of the pharmaceutical formulation. The content of two capsules was evenly distributed along with one of the internal surfaces of a quartz cell and analyzed at various intervals during light exposure until 20 h. The samples for analysis were prepared as described in "Section 2.4".

Degradation under natural light was performed on ethanol solutions with the same concentrations used in the forced degradation.

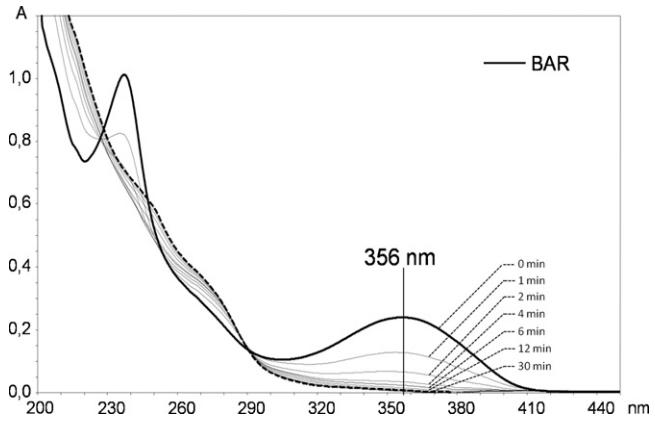

Fig. 1. Spectral sequence of BAR solution 20.0 μg/ml degraded under light at 250 W/m^2.

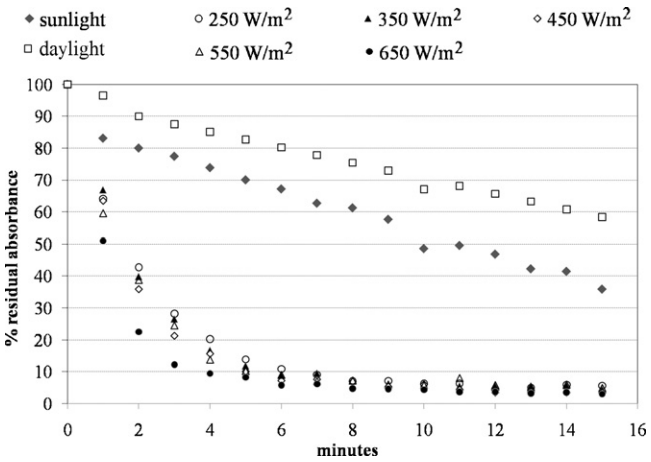

Fig. 2. Photodegradation profiles of BAR under natural light and forced light at different power irradiation.

The samples were exposed to direct sunlight and diffuse light coming from the surrounding sky dome or reflected off adjacent surfaces (daylight). The analyses were made at the following times: 0, 1, 5, 10, 15, 20 and then every 10 min until 100 min.

3. Results and discussion

3.1. Kinetics of BAR photodegradation

In accordance with the recommendations of ICH guideline, a series of ethanol solutions of BAR 20.0 μg/ml were subdued to forced photodegradation under the standard conditions above reported. Fig. 1 shows, as an example, the sequence of spectra collected during a degradation experiment with an irradiation power set at 250 W/m^2.

BAR degradation was monitored through the gradual disappearance of the maximum peak at 356 nm, that is the typical signal of the 1,4-dihydropyridine structure. A contemporary increase of a new peak in the zone 260–280 nm, characteristic of the pyridinic group, demonstrated the oxidation of the dihydropyridinic ring, in agreement with the common degradation occurring for the drugs belonging to this class [7,8]. However, the absence of clear isosbestic points suggested the formation of secondary products. In fact, an isosbestic point should be present whenever there is only a single by-product overlying the parent product. The lack of such a point consequently indicates the existence of multiple by-products [28,29].

A clear influence of the irradiation power on the degradation rate was shown during the first 6 min of irradiation by plotting the residual concentrations of BAR against the degradation time (Fig. 2). After this time, a degradation of about 90% was calculated under whatever irradiation power. The forced tests induced a degradation following a second-order kinetics, with the formation of two or more products [30]. When the values of concentration percentage (BAR%) were plotted against the degradation time, straight lines with slope k_2 and intercept 0.01 were obtained, according to the following equation:

$$\frac{1}{\text{BAR\%}} = k_2.t + 0.01$$

where BAR% is the percentage of the residual drug concentration, k_2 the photodegradation rate constant, t the time (min) and 0.01 is the reciprocal value of the starting concentration percentage (100%). Table 1 lists the parameters describing the photodegradation kinetics for all the samples. The calculated degradation rates resulted higher than those of other congener drugs and comparable to that of Nifedipine, the prototype compound of the 1,4-dihydropyridine class [7].

This relevant sensitivity of BAR to light led us to check the behavior of the drug when exposed under natural lighting. A full knowledge about the intrinsic photochemical characteristics of a so unstable drug seemed in fact essential to set up efficient manufacturing or formulation.

For this aim, two ethanol solutions of BAR 20.0 μg/ml in quartz cells were exposed to direct sunlight and daylight, respectively. The experiments were replicated three times on different days, to take into account the variability of weather, but not significant differences were detected. The concentration of BAR during these degradation tests was plotted against the exposure time, giving the curves reported in Fig. 2. In this case, the degradation process had followed a first-order kinetics, according to the solely formation of the pyridine by-product. The persistence of isosbestic points in both the spectral sequences confirmed this hypothesis. A good linearity was obtained by plotting the logarithm of the residual drug concentration as a function of time, in agreement with the equation:

$$\log \quad \text{BAR\%} = -k_1.t + 2$$

where BAR% is the percentage of residual concentration, k_1 the photodegradation rate constant, t the time (min), 2 the logarithm of the starting concentration (100%). The kinetic parameters describing the degradation process under natural light are also reported in Table 1.

Photodegradation rate of the samples exposed to direct sunlight was significantly higher than the samples kept under daylight. The different degradation pathways that BAR showed when exposed to natural or forced light was confirmed by HPLC-DAD analysis, as described in the next paragraph.

Results obtained from our investigation highlighted a clear disagreement between natural and artificial exposure, making critical

Table 1
Kinetic parameters of photodegradation under different irradiation sources and power values.

Forced irradiation			Natural irradiation		
W/m^2	K_2	R^2	Source	K_1	R^2
250	0.0125	0.9227	Sunlight	0.0236	0.9723
350	0.0130	0.9292	Daylight	0.0150	0.9923
450	0.0189	0.9477			
550	0.0207	0.8925			
650	0.0243	0.9549			

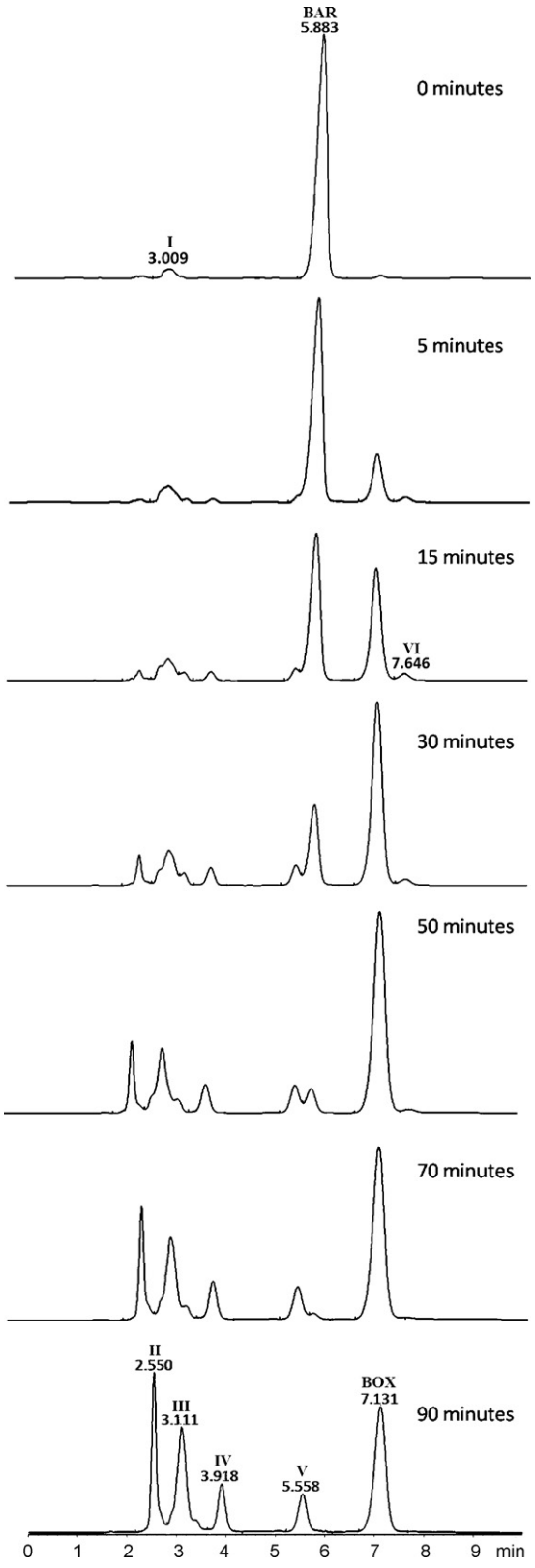

Fig. 3. Chromatograms recorded at sequential times of an ethanol solution of BAR 200.0 μg/ml exposed to stressing light irradiation.

the definition of appropriate analytical methods to be used in the control quality of BAR and its pharmaceutical formulations. These results suggested that data from the forced degradation cannot be automatically extended to degradation occurring under real conditions. The assessment of photostability under natural light seems all the more necessary in the presence of highly photosensitive drugs presenting degradation pathways depending on the light source.

3.2. HPLC study of forced photodegradation of BAR

The forced photodegradation test had revealed a complex degradation process with the formation of several photoproducts, in contrast with the degradation under natural light leading to the pyridine derivative only. Therefore, a HPLC-DAD study was conducted to investigate the pathway occurring in the degradation process. Chromatographic analysis was executed by using the conditions above described in "Section 2.2". The study was performed by exposing an ethanol solution of 200.0 μg/ml BAR to artificial light. The sample was analyzed by HPLC just after the preparation and at different times during light exposure until a total time of 100 min.

A low value of radiant power (250 W/m^2) was chosen to perform the forced degradation study, because of the high sensitivity to light of BAR. The chromatograms recorded at sequential times were assembled in Fig. 3. A very low amount of a secondary product, with *RT* 3.009 (*I*), is already initially present as an impurity of BAR. The absence of any characteristic peak after 210 nm in the UV spectrum of this compound indicated that it did not derive from drug degradation.

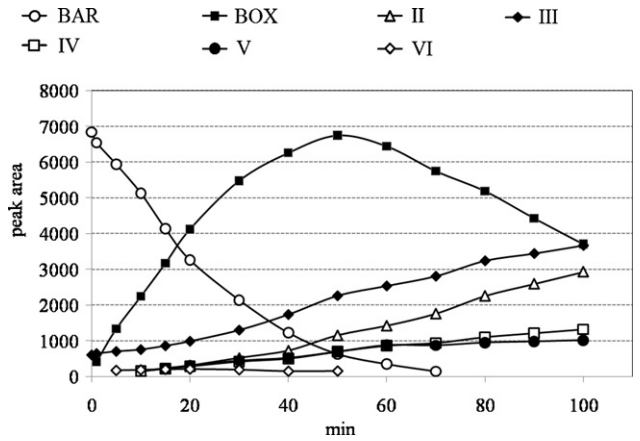

Fig. 4. Kinetic profiles of the products involved in the photodegradation of BAR 200.0 μg/ml exposed to stressing light irradiation.

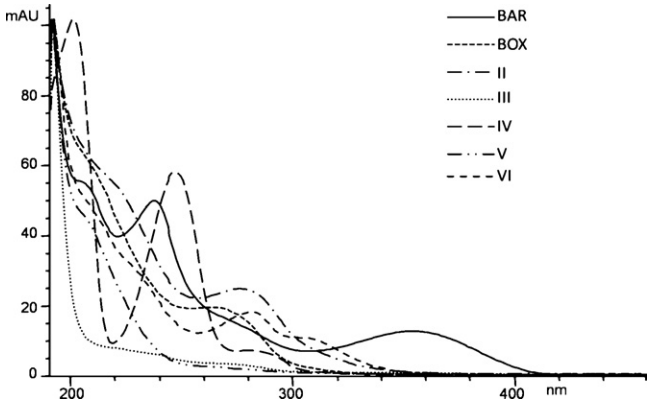

Fig. 5. Pure spectra of BAR and relative photoproducts obtained in the photodegradation of BAR 20.0 μg/ml exposed to stressing light irradiation.

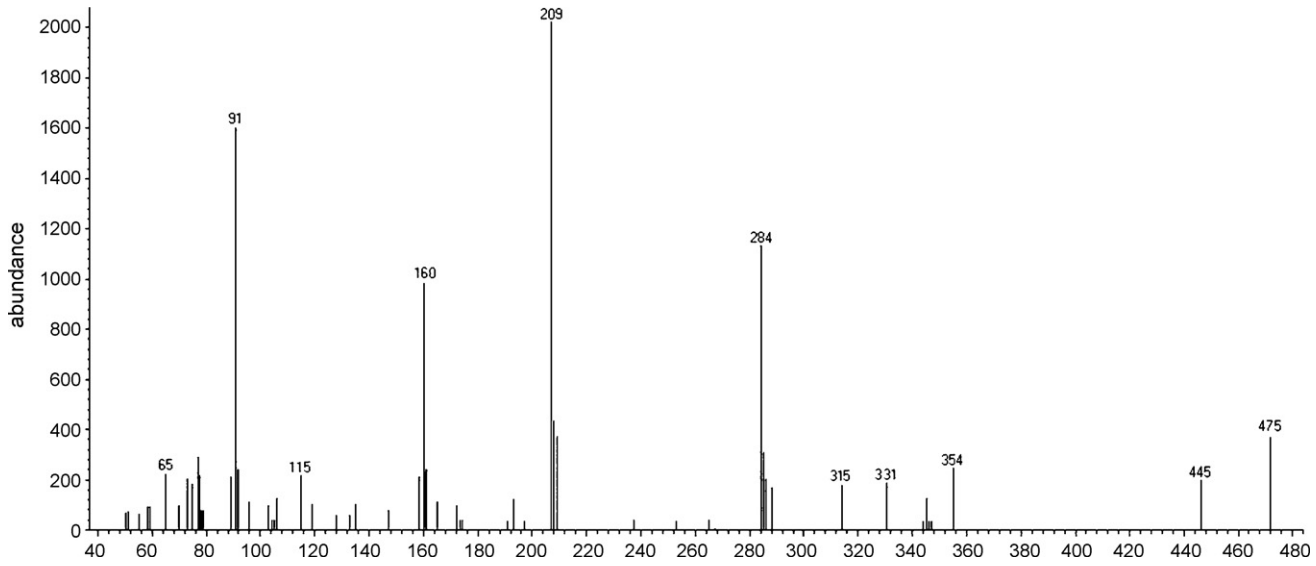

Fig. 6. MS spectrum of BAR.

During the stressing test, the BAR signal (*RT* 5.883) rapidly decreased with the contemporary formation of a first degradation product with *RT* 7.131, corresponding to the pyridine by-product BOX, as confirmed by its UV spectrum. The formation of other five secondary products was evident after only 15 min of light irradiation, positioned at 2.550 (II), 3.111 (III), 3.918 (IV), 5.558 (V) and 7.646 (VI) min, respectively. The graph in Fig. 4 reports the plotting of the chromatographic peak areas for the parent drug and its main photoproducts against time.

It is evident the high photodecomposition rate of BAR, with a halving of its concentration after only 19 min and a complete disappearance after 70 min. The corresponding increase of BOX resulted constant until about 50 min, afterwards it decreased with the simultaneous increase of the secondary degradation products II, III, IV and V. In contrast, the product VI seemed only an intermediate degradation product, as it disappears after about 50 min. These results suggested a first degradation step in which BAR was oxidized to the pyridinic form which in turn underwent a more complex degradation process leading to the formation of different secondary by-products. Effectively, all these secondary photoproducts presented no absorbance above 320 nm, confirming so the saturation of the dihydropyridinic ring in their molecular structures [31,32]. The UV spectra of all the products involved in the chromatographic analysis are shown in Fig. 5.

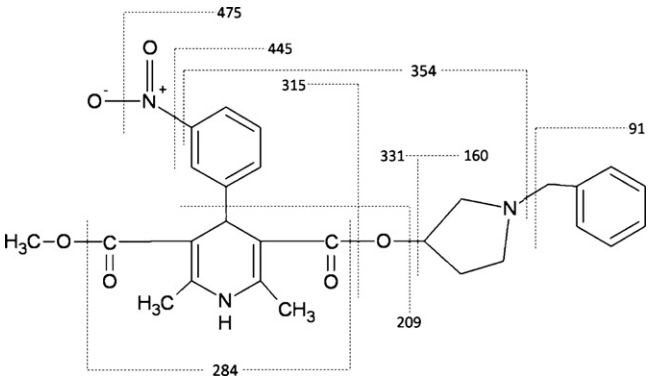

Fig. 7. Scheme of the fragmentation pattern.

The exact identification of the photoproducts was attempted by means of GC–MS analysis, but their low concentration and easy degradability at high temperatures made this study very diffi-

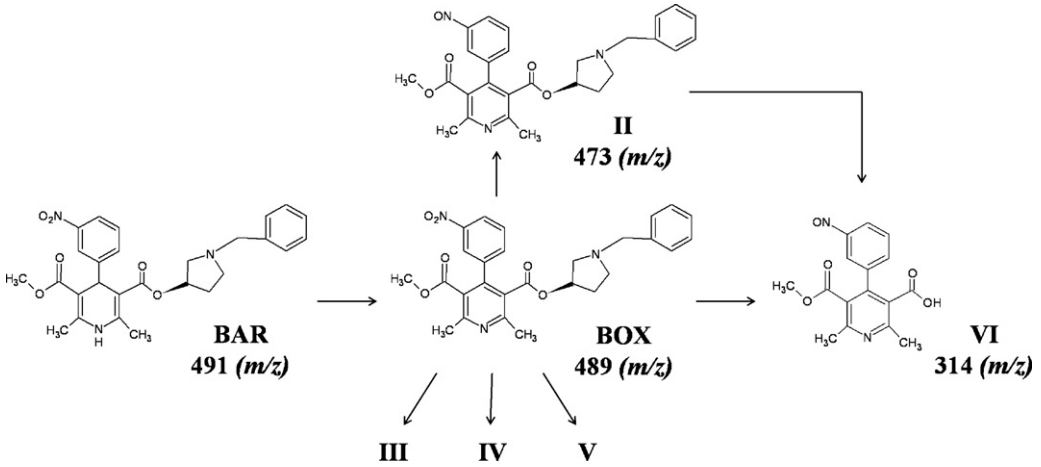

Fig. 8. Scheme of photodegradation pathway.

cult. The photo-exposed solutions of 200.0 μg/ml BAR in ethanol were subjected to GC–MS analysis using the above reported conditions. The mass spectra were recorded providing information on the molecular weight and structure of the analytes.

MS spectrum of BAR and a representative scheme of the fragmentation pattern, are shown in Figs. 6 and 7, respectively. This fragmentation was also found for some photoproducts. The photoreactive centres, on which the mean modifications take place, were found to be the dihydropyridinic ring, the nitro group and the benzylic group on the side chain. In particular, the dihydropyridinic moiety was rapidly oxidized to give the pyridinic by-product (BOX). The subsequent products derived then from further degradation of this molecule, as depicted in Fig. 8. The nitro group was converted in nitroso to furnish the photoproduct II; MS data of the product VI were in accordance with the loss of the benzyl-pyrrolidinic group in the side chain with the contemporary reduction of the nitro group in nitroso. Characterization with the same reliability of the photoproducts III, IV and V products was not possible and their identification is still under investigation. Effectively, these results are in agreement with those already reported in the literature in the photodegradation pathways of some congeners [33,34].

The influence of the nitro group on the photodegradation process is deeply discussed in other studies [7,35]. The probably formation of a metabolite of BAR with loss of the benzylic substituent has been also reported [36].

In contrast, the chromatographic study on the BAR solutions exposed to natural light demonstrated a simplest photodegradation process. Fig. 9 shows the chromatograms recorded at sequential times for the sample kept under daylight. The principal degradation process was the aromatization process of the drug to give the pyridinic photoproduct BOX. The formation of the secondary photoproducts II, III, IV and V, observed during the forced degradation, was detected only after about 1 h of light exposure but their concentration remained under a value of 5–6%, with respect to BAR, throughout the entire experiment. The photoproduct VI was never revealed.

Exposure of an analogous BAR sample to direct sunlight showed the same qualitative results with a moderate increase of the degradation rate.

3.3. Photochemical study

Characterization of a photodegradation process through the study of the transient species, their lifetime and the course between precursor and products, is a crucial way to understand the potential photo-toxicity of a drug and the causes determining it. The transient species can be monitored by using emission (luminescence technique) and absorption (laser flash photolysis) techniques.

The phototoxic effects of 1,4-dihydropyridines are well known [37]. A very important feature of the photodegradation of these drugs involves the probable formation of singlet oxygen that can modify or destroy tissues causing significant damages [38] and loss of therapeutic activity. A contribute to understand the general mechanism of photo-oxidation of Nimodipine and Felodipine has been reported by Pizarro et al. [39] Later, Fasani et al. [40] proposed a general scheme for the photochemistry of 1,4-dihydropyridines. Actually, no photochemical studies are present about the drugs of last generation belonging to this class.

In order to investigate the mechanism of the fast degradation of BAR, the drug was submitted to the steady state photolysis study. BAR solutions (23.8 μg/ml) with maximum absorbance 0.3 were irradiated at a monochromatic light fixed at λ_{exc} 356 nm. The samples were prepared in both ethanol and acetonitrile, but the photoreaction resulted not significantly affected by the medium. The spectra showed the characteristic UV–visible features of the 1,4-dihydropyridines. The generated photoproduct was due to the loss of two hydrogen atoms, in position 1 and 4, of the dihydropyridinic moiety to give a pyridinic by-product.

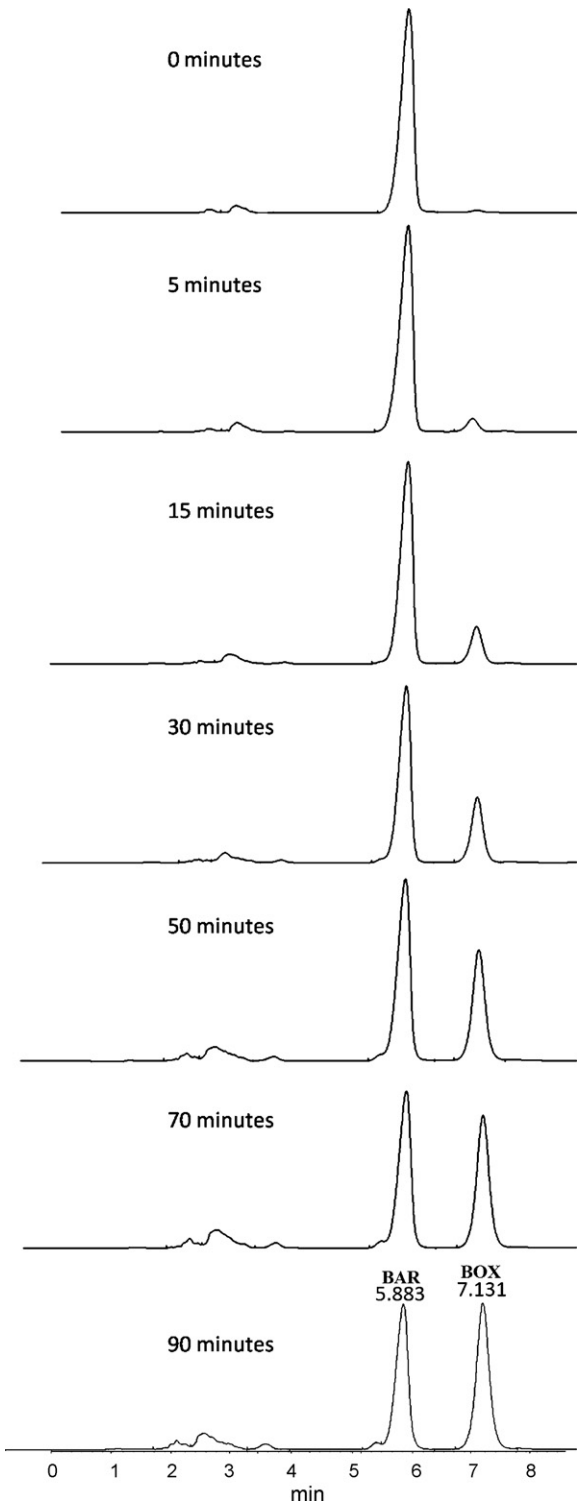

Fig. 9. Chromatograms recorded at sequential times of an ethanol solution of BAR 200.0 μg/ml exposed to daylight.

No detectable fluorescence of BAR due to a singlet excited state was observed at room temperature. This state was probably deactivated by the fast intramolecular electron transfer from the dihydropyridinic ring to the substituted phenyl ring, yielding a zwitterionic radical as an intermediary species. The same mechanism has been proposed for congeners of BAR [40]. Besides, the

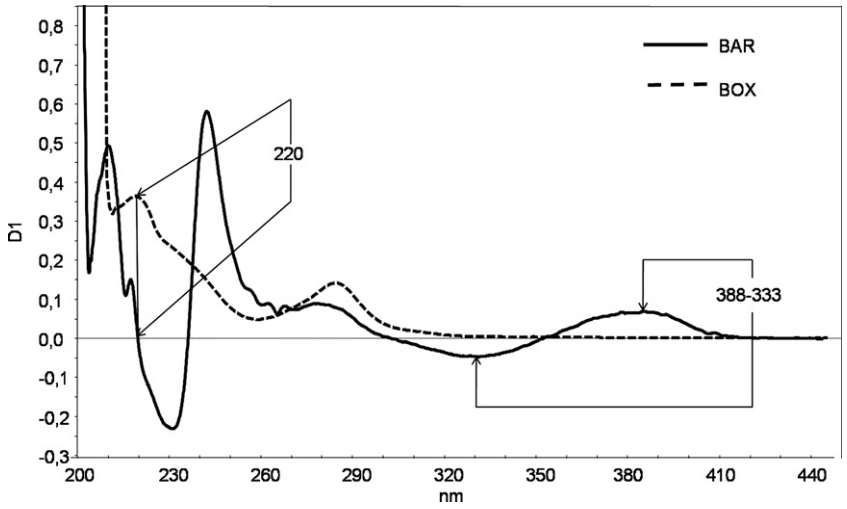

Fig. 10. First-order derivative spectra of BAR and BOX ethanol solutions.

presence of a nitro group on the phenyl ring plays an important role in this mechanism. In fact, it increases the charge delocalization during excitation and promotes the formation of a zwitterionic radical. A 1,4-dihydropyridine drug is so quickly oxidized to pyridinic byproduct thanks to both ionization and deprotonization.

Likewise, a triplet state due to an intersystem crossing (ISC) was not identified. Transition from the initial lowest excited singlet to the triplet state and from the triplet back to the ground state is surely accelerated by the nitro group, in a similar way to many aromatic compounds. This is in agreement with previous studies on other dihydropyridinic drugs [41].

3.4. Assay of BAR and pyridine photoproduct by derivative spectrophotometry

Despite the results obtained from the forced degradation revealed the formation of a large number of products, degradation under natural conditions showed a single product and traces of the others. So a simple and reliable method to be applied in the routine quality control of BAR and its pharmaceutical formulations was defined, able to determine simultaneously the drug and its main pyridinic photoproduct, produced from usual exposure to light.

Actually, BAR concentration resulted proportional to the absorbance measurement of the maximum peak at 356 nm, because of the insignificant absorbance of the degradation product at that wavelength. Unfortunately, the absorption spectrum of a mixture of the two components showed no specific signal for BOX, whose spectrum is even fully overlapped by the BAR spectrum.

Therefore the derivative spectra (first to fourth-order) of the binary mixture were checked to select a suitable spectrum to be used for the simultaneous determination of the components. Derivative techniques in spectroscopy often offer a powerful tool for a resolution enhancement, when signal overlap or interference exists. Several specific signals were singled out for the two components in the spectra of different derivative orders but the first-order derivative spectra seemed to be the most suitable for analytical aim. In Fig. 10, the 1st-order derivative spectra of BAR and BOX, both presenting a 20.0 μg/ml concentration, are plotted.

BAR concentration was carried out by using the peak-through $^1D_{333-388}$ whose absorbance was exclusively due to the drug. Because of the absence of specific signals of BOX, its determination was performed through a zero-crossing approach. This technique states that the absorbance measured to a specific wavelength in a derivative spectrum exactly corresponds to the concentration of one component in a mixture, when the absorbance curves of the remaining components cross the abscissa axis.

Several suitable signals in the BAR–BOX mixture fitted such a requirement. In particular, the spectral line of BAR crossed the *X*-axis at 220, 237 and 304 nm, where the absorbance values reflected the concentration of the photoproduct only. Previous studies reported that the accuracy in a zero-crossing application increased when a signal corresponding to a maximum in the spectrum was used [42]. On the contrary, the results carried out by signals placed on peak shoulders resulted less accurate and precise. BOX concentration was so carried out by considering just the peak at 220 nm that corresponds to a maximum signal of the photoproduct spectrum. Analogous signals could be singled out in higher derivative order spectra, but the rising of the instrumental noise prevented an accurate analysis.

The equation parameters calculated for determination of BAR or BOX concentration (independent variables) are reported in Table 2. These relationships were applied to assay the samples of the prediction set.

An independent HPLC-DAD method was simultaneously developed to check the results obtained by the spectrophotometric analysis. Calibration curves for the BAR and BOX were constructed in the range 50.0–400.0 μg/ml and obtained by applying a least squares linear regression to the concentration values carried out by chromatograms at the retention time (RT) of 5.98 and 7.08 min, respectively. The relative equation parameters, with BAR or BOX

Table 2
Equation parameters and statistical results for determination of BAR and BOX, as independent variables.

Analytical method	Analyte (μg/ml)	Signal Abs/Area	Slope	Intercept	R^2	% Recovery	RSD
Zero-order derivative	BAR	356 nm	0.0130	−0.0095	0.9989	98.37	1.46
First-order derivative	BAR	388–333 nm	0.0058	−0.0031	0.9989	97.56	2.18
First-order derivative	BOX	220 nm	0.0185	−0.0203	0.9984	96.11	3.22
HPLC	BAR	5.98 min	33.958	6.0248	0.9969	97.67	2.52
HPLC	BOX	7.08 min	47.800	56.783	0.9999	96.43	3.84

concentrations as independent variables, are reported in Table 2. Results from application of both UV derivative and HPLC methods were collected in terms of accuracy (% recovery) and precision (relative standard deviation RSD) and listed in Table 2.

The content of BOX in raw material was found to be approximately 3% after 8 h under daylight. The pharmaceutical formulations of BAR were analogously tested but they showed high stability under both natural and stressing conditions. The data were carried out from three replicate analyses and very low variance was measured in all experiments. In particular the pharmaceutical capsules showed a decrease of BAR concentration of 3.2% and 5.4% after 38 h of exposure to natural and artificial light, respectively. The capsules resulted well protected when enwrapped by the packaging material.

4. Conclusions

The proposed work demonstrated the difference in the photodegradation pathway occurred when the antihypertensive barnidipine is exposed to natural or stressing irradiation. A HPLC procedure was developed to investigate the degradation processes. The drug, with a feature common to all compounds of the dihydropyridinic class, when exposed to sunlight or daylight was oxidized to the corresponding pyridinic derivative. In contrast, exposure to stressing light by Xenon lamp caused a more complex photodegradation process with the formation of several photoproducts in addition to the pyridinic by-product. In this case, BAR was subjected to the aromatization of the 1,4-dihydropyridine moiety, reduction to the nitroso-derivatives and N-debenzylation on the side chain as major photodegradation reactions.

A method for the simultaneous determination of BAR and pyridinic photoproduct, produced from usual exposure to light, was developed by derivative spectrophotometry, useful as a routine quality control of the drug formulations. The whole procedure required only a simple and rapid sample preparation with a negligible light exposure when applied to pharmaceuticals. The spectrophotometric results were confirmed by HPLC as a reference procedure. The results reported in this work could be useful to identify precautionary measures needed in manufacturing or in formulation of the drug products.

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