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Journal of Pharmaceutical and Biomedical Analysis
32 (2003) 929–935
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Photochemical degradation of solid-state nisoldipine monitored by HPLC

Valentina D. Marinkovic [a], Danica Agbaba [b,*], Katarina Karljikovic-Rajic [b], Sote Vladimirov [b], Jovan M. Nedeljkovic [c]

[a] *Zdravlje Pharmaceutical and Chemical Industry, Quality Control Sector, 16000 Leskovac, Serbia and Montenegro, Yugoslavia*
[b] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, P.O. Box 146, 11000 Belgrade, Serbia and Montenegro, Yugoslavia*
[c] *Vinca Institute of Nuclear Sciences, P.O. Box 522, 11001 Belgrade, Serbia and Montenegro, Yugoslavia*

Received 24 April 2002; received in revised form 27 February 2003; accepted 28 February 2003

Abstract

The photochemical degradation of solid-state nisoldipine, 1,4-dihydropyridine calcium antagonist, was investigated under daylight and UV light conditions. Degradation products were identified by using the retention times of corresponding standards and quantified by high-performance liquid chromatographic method. The daylight illumination induced appearance of nitrosophenylpyridine, while formation of second degradation product, nitrophenylpyridine, was observed only upon UV light illumination. The photodegradation kinetics of solid-state nisoldipine under daylight and UV light illumination belongs to class of zero-order reactions. The rate constants of disappearance of nisoldipine upon illumination were determined for raw material as well as pharmaceuticals (tablets, film-tablets and capsules).

Keywords: Nisoldipine; Photochemical degradation; Illumination

1. Introduction

Nisoldipine, (±)3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2′-nitrophenyl)-pyridine-3,5-dicarboxylate, an orally active calcium blocking agent belonging to the dihydropyridine family with nonsymmetrical esters functions, is indicated in the treatment of angina pectoris, hypertension and congestive heart failure [1].

The most undesirable property of dihydropyridines, from a pharmaceutical point of view, is high photochemical sensitivity, which can induce molecular changes leading to decrease of therapeutic effect and even some toxic effects after i.p. administration [2]. The photo-induced changes of 1,4-dihydropyridines involve the oxidation of the

* Corresponding author.
E-mail address: dana@yubc.net (D. Agbaba).

0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00194-8

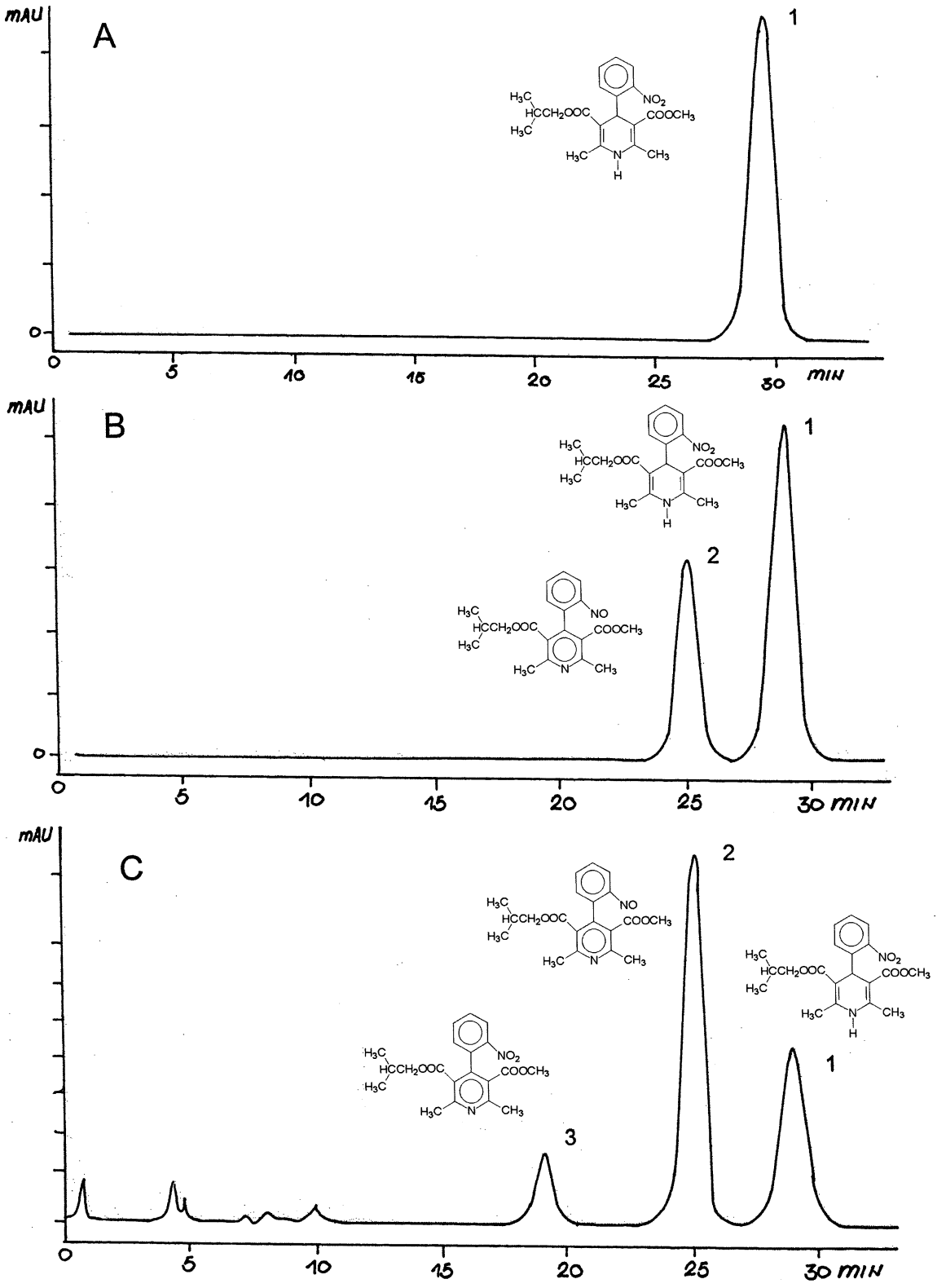
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Fig. 1.

dihydropyridine ring to a pyridine ring and the reduction of the aromatic nitro group to a nitroso group [3–6]. Stability of nisoldipine [7,8], nifedipine and other 1,4-dihydropyridine derivatives [9,10] in organic solvents and in inclusion complexes with β-cyclodextrin have been studied by GC and dual-wavelength UV spectroscopic techniques. Ragno et al. reported the third order UV derivative spectroscopic methods for determination of nitrendipine [11], amlodipin [12] and their photodegradation product. The degradation of nisoldipine in ethanol exposed to UV light was investigated by means of high-performance liquid chromatography (HPLC) and MS [2]. These authors isolated and identified two additional degradation products (dimmers of the nitroso derivative) besides typical degradation products of 2-nitro-1,4-dihydropyridines (nitroso and nitrophenylpyridine derivatives).

The electrochemical study of degradation of nisoldipine and its pharmaceutical forms in ethanol under two different types of illumination conditions (artificial daylight and UV light) has been reported [13]. Recently, hydrolytic degradation of nitrendipine and nisoldipine carried out at different pHs and temperatures has been reported [14]. Also, degradation of solid-state nisoldipine under illumination conditions mimicking daylight was studied in our laboratory by first and second order UV derivative spectroscopy [15]. Molecule structure of nisoldipine and its degradation products had been proceeded in our previous investigations [16,17].

In this paper, we are focused on the kinetics of degradation of solid-state nisoldipine and its pharmaceutical forms upon exposure to either artificial light mimicking daylight conditions or UV light. Also, main photodegradation products were identified using retention times of corresponding standards and HPLC method was validated for their quantification in raw nisoldipine and pharmaceuticals.

2. Experimental

2.1. Materials

Nisoldipine and its degradation products (nitroso and nitro analogues) standard substances were obtained from Promed (Praha, Czech Republic) and were used as received. Nisoldipin 5 and Nisoldipin 10 tablets were obtained from Slaviamed (Belgrade, Yugoslavia), while Nisoldipine capsules were prepared in Zdravlje (Leskovac, Yugoslavia). Methanol and ethanol, were HPLC grade (Merck, Darmstadt, Germany) water purified by a Millipore Milli-Q system was used for the preparation of solutions. Phosphoric acid were obtained from Merck, Darmstadt, Germany.

2.2. Apparatus

For UV illumination, a GL 15 lamp (15 W Germicidal, Japan) was used. For artificial-daylight illumination, a 200 W Phillips tungsten lamp was used.

HPLC experiments were carried out on a Hewlett Packard LC 1100 instrument, equipped with binary solvent pump G 131 2A and variable UV detector G 1314A. An octadecylsilane column (Lichrosorb RP-18, 5 μm, 250 × 4 mm, Merck, Darmstadt, Germany) was used and methanol–water (60:40 v/v), pH 3.0 adjusted with phosphoric acid was used as mobile phase. Flow rate 1 ml/min and loop 20 μl were used. The samples were monitored at 238 nm.

2.3. Preparation of test and reference solutions

Stock solutions of nisoldipine and its nitroso and nitro analogues were prepared in methanol (0.2 mg/ml). Series of five standard solutions of nisoldipine and its nitroso and nitro analogues were prepared by diluting the corresponding stock solution (10–100 μg/ml for nisoldipine, and 1–100 μg/ml for nitro and nitroso analogues).

Fig. 1. Chromatograms of nisoldipine: (A) before illumination, (B) upon artificial daylight illumination, and (C) upon UV illumination. Peaks 1, 2 and 3 correspond to nisoldipine, nitroso and nitro derivative, respectively. Structural formula of nisoldipine and its degradation products is also included.

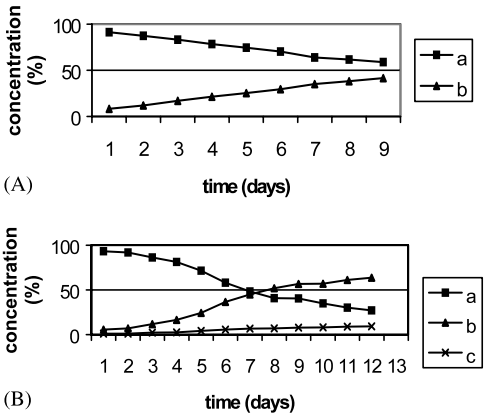

Fig. 2. Photodegradation kinetics of nisoldipine: (A) under artificial daylight illumination, and (B) under UV light illumination. Curves a–c correspond to nisoldipine, nitroso and nitro derivatives, respectively.

2.4. Sample preparation

Solid state nisoldipine samples for UV or artificial daylight illumination experiments were in the form of compact layer (<1 mm) on the bottom of Petri dish. These samples were obtained by evaporating methanol from the nisoldipine solution of appropriate concentration, and consequent drying under vacuum. Samples containing mixture of raw nisoldipine and lactose in different ratios (100, 80, 60 and 40 wt.% of nisoldipine) were prepared on the same manner. These samples were exposed to the light source (UV or artificial daylight) up to 10 days (distance between light source and sample was 8 cm). Nisoldipine tablets, film-tablets and capsules were kept in glass containers and exposed to the light under above described conditions.

In order to follow kinetics of degradation of nisoldipine upon illumination by HPLC, on a daily basis 10 mg of illuminated sample was taken out and dissolved in methanol (final concentrations were 0.1 mg/ml). Obtained results are the average values of three series of testing under the same experimental conditions.

3. Results and discussion

3.1. HPLC analysis

Chromatographic separation of nisoldipine from its degradation products is shown in Fig. 1. The appearance of two photodegradation products of nisoldipine (1) dissolved in ethanol after exposure to artificial light can be noticed. One is nitrosophenylpyridine (2), whose appearance was induced by daylight illumination, while the other one, nitrophenylpyridine (3), appeared only upon UV illumination. Resolution and selectivity of chosen method is satisfactory (retention times of nisoldipine, nitroso and nitro analogues are 29.2, 25.0 and 19.8 min, respectively). These retention times are sufficiently different to obtain an independent quantitative analysis of nisoldipine and its photodegradation products. The shorter retention times for photodegradation products compared to the retention time for nisoldipine are due to more polar character of degradation products. Previously reported dimmers of the nitroso derivative [4] were not detected in our experiments, most likely because power of the UV lamp was low (15 W).

The response (peak area) was proportional to the concentrations over the tested range (10–200 μg/ml for nisoldipine, and 1–200 μg/ml for nitroso and nitro analogues). The detection limits for nitroso and nitro analogues were 0.3 and 0.4 μg/

Table 1
HPLC analysis of nisoldipine raw material, Nisoldipin 5 and Nisoldipin 10 tablets

Sample	Declared, nisoldipine (mg)	Assay (mg ± R.S.D.)		
		Nisoldipine	Nitroso	Nitro
Raw material	10	9.98 ± 0.154	0.05 ± 0.0004	–
Nisoldipin 5	5	5.12 ± 0.102	0.04 ± 0.0001	–
Nisoldipin 10	10	10.02 ± 0.201	0.06 ± 0.0007	–

ml, respectively, defined as the analyte concentration giving a signal equal to three times the standard deviation of the blank signal.

The following regression equations were obtained:

$y = (63.502 \pm 2.22)x - (316.46 \pm 9.31)$;

$r = 0.9981 (n = 5)$ for nisoldipine,

$y = (35.865 \pm 1.12)x + (8.284 \pm 0.33)$;

$r = 0.9999 (n = 5)$ for nitroso analogue,

and

$y = (34.725 \pm 1.31)x - (43.646 \pm 1.76)$;

$r = 0.9986 (n = 5)$ for nitro analogue.

The repeatability of analytical system was determined by using two samples of nisoldipine that contained 0.1 and 0.5 wt.% of nitroso derivative. Six consecutive replicate injections of each sample gave R.S.D. of 2.3 and 1.2%, respectively. The repeatability of nisoldipine assay was checked with concentrations of 0.1 and 0.2 mg/ml, and R.S.D. values were found to be 1.3 and 0.6%, respectively.

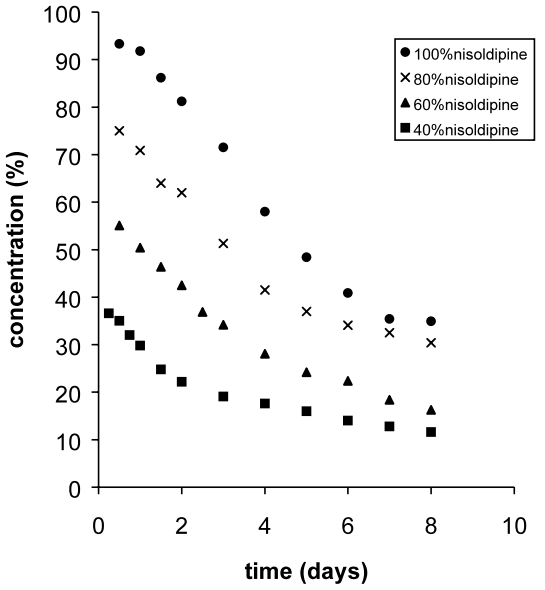

Fig. 3. Photodegradation kinetics of nisoldipine in the mixture with lactose (100, 80, 60 and 40 wt.% of nisoldipine) under UV light illumination.

The accuracy of the method was checked using 0.2 mg/ml solution of nisoldipine with no detectable presence of degradation products. The solution was then spiked with nitroso analogue (0.5 and 2 wt.%). The obtained recoveries of 102.5 and 100.5% for nitroso analogue indicated adequate accuracy of the method.

The robustness study of the suggested HPLC method included evaluation of influence of the composition of mobile phase (water–methanol mixture) as well as detection wavelength on chromatographic behavior of tested substances. It was found that content of methanol lower than 60% lead to deformation of chromatographic peaks (nonsymmetrical peaks with tails), while overlapping of chromatographic peaks was noticed when content of methanol was larger than 65%. Optimum pH was 3, because higher pH provided lower sensitivity of the method and at wavelength between 238 and 250 nm sensitivity would be also lower.

The applicability of the suggested method was tested by analyzing three different kinds of samples: raw material, Nisoldipin 5 and Nisoldipin 10 tablets. In Table 1 are presented the average results obtained from these measurements. It should be pointed out that all values are within limits declared by the supplier.

3.2. Photochemical degradation

In order to determine influence of different spectral regions of light on degradation of solid nisoldipine, photochemical experiments were performed using artificial light sources with different spectral characteristics (light source mimicking daylight conditions with dominant emission in VIS spectral region and UV light source).

The kinetic data for photodegradation of solid nisoldipine under daylight conditions are presented in Fig. 2A. Under these experimental conditions, HPLC measurement indicated that nitroso derivative is the only photodegradation product, and kinetics of its formation is also shown in Fig. 2A. The photodegradation kinetics of nisoldipine belongs to class of zero-order reactions, and under stated experimental condi-

tions the rate constant was found to be 4.3 wt.%/day.

The kinetic data of the degradation of solid nisoldipine under UV light illumination are shown in Fig. 2B. The UV light induced more complex photodegradation kinetics compared to artificial daylight conditions, since formation of both photodegradation products, nitroso and nitro derivatives, took place. The kinetic data of the formation of photodegradation products are also included in Fig. 2B. It is clear that kinetics of degradation of solid nisoldipine under UV light illumination belongs to the class of consecutive chemical reactions because UV light is sufficiently energetically reach to initiate transformation of nitroso derivative into nitro derivative. Also, disappearance of nisoldipine under UV light is linear function of time until the reaction is 60% completed. After that the photodegradation kinetics is slowed down. This observation is similar to the data reported by Marciniec and Rychcik [18] and Majeed et al. [19]. It should be noticed that photodegradation of nisoldipine is faster under UV light illumination compared to daylight conditions. The rate constant determined from the linear part of kinetic curve was found to be 10.0 wt.%/day.

In order to clarify order of reaction for photochemical degradation of solid nisoldipine under UV light illumination the additional kinetics experiments were performed. The samples prepared with lactose and containing different weight percentages of nisoldipine (100, 80, 60 and 40 wt.%) were exposed to UV light under identical experimental conditions. The kinetic curves of disappearance of nisoldipine are shown in Fig. 3. It should be noticed that times $\tau_{0.1}$ and $\tau_{0.5}$ did not remain constant for different initial concentrations of nisoldipine, as would be expected from a first-order reaction. On the contrary, the ratio between $\tau_{0.1}$ times is exactly the same as the ratio between initial concentrations of nisoldipine, what is typical for zero-order reactions. The slower photodegradation kinetics manifested by deviation from linearity of kinetic curves can be observed at longer times. It is obvious that gradual attenuation of the light intensity takes place in the bulk of the sample in direction from front to back side of the sample. Because of that, at longer illumination times when most of nisoldipine in the front part of the sample is transformed in its degradation products the kinetics is slowed down because nisoldipine in the back part of the sample experience lower light intensity. In the ideal experiment the photodegradation kinetics should be studied by illuminating monomolecular layer of nisoldipine. Our preliminary experiments indicated the increase of linear region by the decrease of thickness of nisoldipine layer.

Pharmaceuticals (nisoldipine tablets, film-tablets and capsules) were also exposed to UV light under identical experimental conditions. The rate constants of nisoldipine disappearance were determined from the linear part of kinetic curves (6.9, 1.9 and 1.1 wt.%/day for nisoldipine tablets, film-tablets and capsules, respectively). It should be noticed that capsules provide the best protection against UV light because photodegradation of nisoldipine in this pharmaceutical form is over six times slower compared to tablets.

Acknowledgements

Financial support for this study was granted (Project No 1458) by the Ministry of Science, Technology and Development of the Republic of Serbia.

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