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RESEARCH PAPER

Spectroscopic and HPLC Studies of Photodegradation of Nilvadipine

Włodzimierz Augustyniak,[1] Jadwiga Mielcarek,[2,*] Marek Milewski,[1] and Olga Szamburska

[1]*Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland*
[2]*Department of Inorganic and Analytical Chemistry, K. Marcinkowski University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland*

ABSTRACT

Photochemical decomposition of nilvadipine (NV), a derivative of 1,4-dihydropyridine (DHP), was studied. Photodegradation was carried out in the conditions recommended in the first version of the document issued by the International Conference on Harmonization (ICH), currently in force in the studies of photochemical stability of drugs and therapeutic substances. Methanol solutions of NV were irradiated with a high-pressure mercury arc lamp, type HBO 200 (300–400 nm). The maximum absorption of radiation at 365 nm was achieved by applying the interference filter and Wood's filter. The assessment of NV photodegradation was made on the basis of the UV spectrophotometric and high-performance liquid chromatographic (HPLC) methods. Quantitatively, the process was described with the calculated rate constants of decomposition k, *time of decomposition of 50% of the compound* $t_{0.5}$, *and time of decomposition of 10% of the compound* $t_{0.1}$. *The two methods applied allowed a determination of the kinetic parameters of NV photodegradation from the relationship* $ln\ c = f(t)$. *Using the Reinecke salt as a chemical actinometer, apparent quantum yields of photodegradation were obtained; after extrapolation to the time of irradiation zero, these gave the actual quantum yield* ($\Phi = 7.3 \cdot 10^{-5}$). *The quantum yield of fluorescence at* $\lambda_{exc} = 375$ *nm was about* $9.3 \cdot 10^{-4}$. *The methods used for evaluation of NV photodegradation were subjected to validation, and*

*Corresponding author. E-mail: jmielcar@mail.usoms.poznan.pl

results of the analytical methods were statistically assessed by Snedecor F *and Student* t *tests. The former test revealed no statistically significant difference between the variances obtained by the HPLC and UV spectrophotometric methods. Also, verification of the zero hypothesis of the Student* t *test on equality of means of the results obtained gave no significant differences between the two methods.*

Key Words: *1.4-Dihydropyridine derivatives; Nilvadipine; Photodegradation; Validation*

INTRODUCTION

The mechanism of nilvadipine (NV) activity, similar to that of the other 1,4-dihydropyridine (DHP) derivatives, involves blocking of the free calcium channel (1). Administration of NV reduces the tension of the smooth muscles of the blood vessels, causing vasodilation. When compared to other DHP derivatives (e.g., nifedipine), NV shows 9–10 times more selective activity toward vessels (2). The studies carried out so far have not revealed its negative ino-, chrono-, and dromotropic activity (3–6).

Like the other DHP derivatives, after oral administration, NV undergoes decomposition according to a first-order reaction. That is why its biological availability is only 14%–19%, and the maximum concentration in blood plasma is reached after about 40 min (7).

From the pharmaceutical point of view, a serious disadvantage of NV is its high photosensitivity (8–11). After exposure to light, NV undergoes irreversible structural changes, leading to a weakening or cessation of the desired pharmacological activity. Assessment of photostability of therapeutic substances and drugs, DHP included, is a complex problem since no standard analytical procedure has been developed for this kind of study. For many years, efforts have been made to find a test for photostability that would establish in a simple way whether a given drug or therapeutic substance is stable in the assumed model of exposure to light (12).

A very helpful document prepared by the Expert Working Group (EWG) of the International Conference on Harmonization (ICH) recommends evaluation of photostability of drugs by the methods described in the two versions (13). The earlier studies on photodegradation of drugs proved that the effect of room illumination by both natural and artificial light should be taken into account, and the effect of high-energy ultraviolet (UV) light can be disregarded. In view of the increasing application of DHP derivatives in medical treatment, studies of their photostability are still of interest.

The aim of this work was assessment of the photodegradation of NV performed following the recommendations of the first version of the ICH document.

EXPERIMENTAL

Apparatus

Liquid chromatography was performed with a Hewlett-Packard 1050 with a UV detector ($\lambda =$ 377 nm), a Jasco type V-550 spectrophotometer, a Specord M 40 UV-Vis spectrometer from Carl Zeiss Jena, and a high-pressure UV lamp with an HBO 200 mercury burner.

Materials

Materials used were nilvadipine, which is the 5-isopropyl-3-methyl ester of 1,4-dihydro-2-cyano-4-(3-nitrophenyl)-6-methyl 3,5-pyridinedicarboxylic acid ($C_{19}H_{19}N_3O_6$), AN 1546 Klinge Pharma; and acetonitrile, methanol, and hexane (Baker) for high-performance liquid chromatography (HPLC).

Irradiation Conditions

A methanol solution of NV at a concentration of $1.0 \cdot 10^{-4}$ mol/L was placed in a cylinder quartz cell of 2.4 ml in volume and $l = 1$ cm in length and then irradiated from a source at a distance of 30 cm. The maximum intensity of the radiation of $\lambda = 365$ nm

was obtained using the interference filter and Wood's filter.

Quantum Yield of Nilvadipine Photodegradation

Quantum yield of NV photodegradation was determined using the Reinecke salt as a chemical actinometer. The NV solutions were irradiated to obtain 60% conversion (% K), determined from a difference in the absorbance measured before and after a given irradiation time. The quantum yield for a given percentage of conversion was calculated from the formula

$$\Phi = \frac{\Delta c \cdot N_A}{I_{\text{abs}} \cdot t}$$

where $\Delta c \cdot N_A$ is the difference in the number of NV molecules in the solution before and after the irradiation, I_{abs} is the intensity of radiation absorbed by the sample, and t is time (seconds).

The obtained dependence of the percentage of NV conversion on the time of irradiation and the quantum yield are given in Table 1. To get real quantum yields, the yields established in the experiment for different times of conversion (% conversion) were extrapolated to the initial intensity of NV (0% of conversion). The values of the real quantum yields obtained by the spectrophotometric method and HPLC were $7.4 \cdot 10^{-5}$ and $6.9 \cdot 10^{-5}$, respectively.

Table 1

Quantum Yield of Nilvadipine Photodegradation ($\lambda_{exe} = 365$ nm) by Spectrophotometric (SP) and High-Performance Liquid Chromatographic (HPLC) Methods

Lp.	Photodegradation Time (min)	Quantum Yield (Φ 10^5)	
		SP	HPLC
1	0	—	—
2	20	6.796	6.573
3	60	7.351	6.999
4	100	7.332	7.241
5	140	7.387	7.188
6	260	6.990	7.047
7	350	6.980	7.119
8	450	6.481	6.881
9	570	5.976	6.211
10	690	5.591	6.044
11	780	5.316	6.043

Quantum Yield of Fluorescence

For the quantum yield of fluorescence (14), the UV-Vis absorption measurements were carried out on a Jasco model V-550 spectrophotometer and on an M-40 Specord (Carl Zeiss, Jenna), whereas emission measurements were performed on a modified and computerized MPF-3 spectrophotometer. The background due to solvent impurities and scattering was subtracted from the emission of a sample. To avoid overestimation of Raman scattering in a sample, which is lower than in the pure solvent, the contribution due to the Raman scattering in the solvent was multiplied by transmittance of the sample for the excitation wavelength. Quantum yield of fluorescence was measured by the relative method using quinine sulfate in 0.05 mol/L sulfuric acid ($\phi_F = 0.52$).

NV shows relatively weak fluorescence ($\phi_{\text{fl}} = 9.3 \cdot 10^{-4}$, $\lambda_{\text{exc}} = 375$ nm) with emission intensity maximum at 520 nm.

Analysis of the Photodegradation Products

Spectrophotometric Method

The NV solution was irradiated for 780 min, and at certain time intervals, UV spectra were taken in the range 200–400 nm. The absorbance of the methanol solutions of NV placed in cells 1 cm long was measured at $\lambda = 367$ nm against methanol as a reference substance (see Fig. 1).

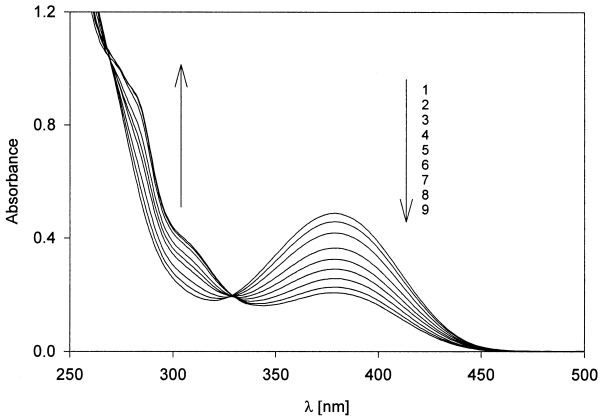

Figure 1. Spectral changes on irradiation of methanol solution of nilvadipine ($c = 35$ μg/ml). Irradiation times (min): 1, 0; 2, 60; 3, 140; 4, 260; 5, 350; 6, 450; 7, 570; 8, 690; 9, 780. $\lambda_{\text{exc}} = 365$ nm, $l = 1$ cm.

High-Performance Liquid Chromatographic Analysis

The HPLC measurements were performed with a 100 RP-18 LiChrospher column (5 μm), which was 200×4 cm in size; the mobile phase was a solution of methanol:water:acetonitrile at a concentration of 42 : 33 : 25 (v/v/v); the volume of the sample deposited was 20 μl; and the mobile phase flow rate was 1 ml/min. Exemplary changes in the HPLC chromatograms observed as a result of NV photodegradation are illustrated in Fig. 2.

Quantitative Evaluation of the Photochemical Process

The photochemical decay of NV was expressed as a dependence of the logarithm of absorbance or the peak area on the time of the exposure of the solutions to light, as in the formula

$$\ln A = \ln A_o - k \cdot t$$

where A is the NV absorbance after a certain time of exposure, A_o is the NV absorbance before the exposure, k is the photodegradation rate constant, and t is time (seconds).

The results obtained by the two methods were used for the calculation of the rate constants of photodecomposition k, time of decomposition of 50% of the compound $t_{0.5}$, and time of decomposition of 10% of the compound $t_{0.1}$ in the first-order reaction.

The kinetic parameters of NV photodegradation obtained by the two methods are given in Table 2.

Validation

Linearity

For validation (15), the linear regression analysis of NV was performed by plotting the absorbance (spectrophotometric method) and the peak area y versus NV concentration x in moles/liter. The calibration curve was constructed in the range, and the following equations were obtained:

Spectrophotometric method

$$\sqrt{1.79 \cdot 10^{-5} \div 9.24 \cdot 10^{-5}} \text{mol/L}$$

$$y = 5.24 \cdot 10^3 x + 0.0034 \qquad r = 0.998$$

Table 2

Kinetic Parameters of Photochemical Decomposition of Nilvadipine from Spectrophotometric (SP) and High-Performance Liquid Chromatographic (HPLC) Methods

Method	Correlation Coefficient r	k (min-10^3)	$t_{0.5}$ (min)	$t_{0.1}$ (min)
SP	0.9999	1.1179	619.90	94.28
HPLC	0.9989	1.2384	559.60	85.11

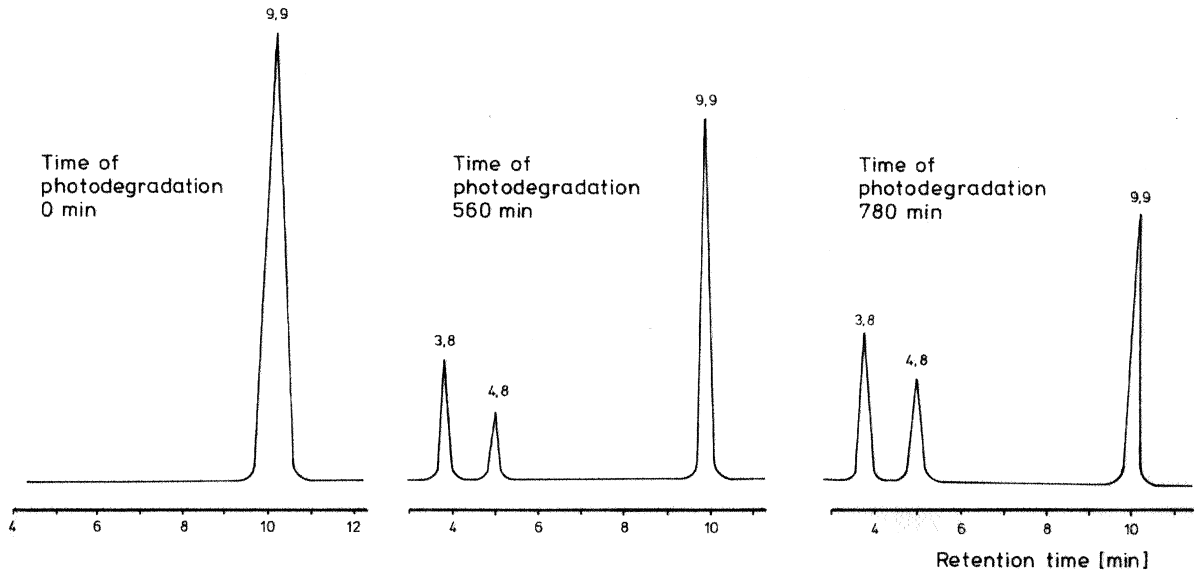

Figure 2. Chromatograms of nilvadipine and of the photodegradation products.

HPLC $\surd 3.35 \cdot 10^{-5} \div 7.99 \cdot 10^{-5}$ mol/L

$$y = 2.40 \cdot 10^{10}x - 5.13 \qquad r = 0.999$$

Detectability

The limits of detection (LOD) and limits of quantification (LOQ) for the two methods were calculated from the regression lines. The following values were obtained. For HPLC,

$\text{LOD} = 0.83 \cdot 10^{-7}$ mol/L and

$\text{LOQ} = 0.40 \cdot 10^{-8}$ mol/L

For the spectrophotometric method,

$\text{LOD} = 6.66 \cdot 10^{-7}$ mol/L and

$\text{LOQ} = 3.57 \cdot 10^{-8}$ mol/L

Precision

The precision of the HPLC method was evaluated on the basis of eight determinations of the concentration of NV in the methanol solution at a concentration of $3.35 \cdot 10^{-5}$ mol/L. The precision of the spectrophotometric method was evaluated from the measurements of absorbance of the NV solution at a concentration of $5.22 \cdot 10^{-5}$ mol/L. Prior to this evaluation, the significance of the results was verified by the Q-Dixon test. The parameters characterizing the precision of the HPLC and the spectrophotometric methods are given in Table 3.

Table 3

Parameters Characterizing the Precision of Nilvadipine Determination by the Spectrophotometric (SP) and High-Performance Liquid Chromatographic (HPLC) Methods

	Method	
Parameter	HPLC	SP
N	8	8
X	$3.2387 \cdot 10^5$	$5.2212 \cdot 10^5$
S^2	$1.8393 \cdot 10^{14}$	$1.4411 \cdot 10^{13}$
S	$1.3562 \cdot 10^7$	$3.7961 \cdot 10^7$
S_y	$4.1874 \cdot 10^3$	$7.2705 \cdot 10^3$
S_x	$4.7949 \cdot 10^8$	$1.3421 \cdot 10^7$
M	$1.13399 \cdot 10^7$	$3.17416 \cdot 10^7$
W_z (%)	0.4687	0.7270

Statistical Comparison of the Results Obtained by the High-Performance Liquid Chromatographic and the Spectrophotometric Methods

The results obtained by the two methods were compared using the Snedecor F test of conformity of variances and Student t test of the significance of the difference between the means.

Regarding different NV concentrations in the solutions used for the spectrophotometric analysis and HPLC, for statistical analysis, the NV concentrations were expressed as relative values according to the formula

$$X_w = \frac{X_i}{X}$$

where X_w is the relative value, X_i is the individual result, and X is the arithmetic mean of X_i.

Snedecor F Test

The conformity of variances was checked by the Snedecor F test, calculating the estimators of variances S_1^2 and S_2^2 from the samples of $n_1 = n_2 = 8$, for the results obtained with the two methods. To verify the zero hypothesis of the Snedecor F test on the identity of two variances at a certain level of significance, it was assumed that $S_1^2 > S_2^2$, and the values obtained were compared with the critical values of $F\alpha_{(n1\text{-}1,\ n2\text{-}1)}$ from the tabulated Snedecor F distribution.

Student t Test

To verify the zero hypothesis of the Student t test on the identity of two means, obtained by the two methods analyzed, the value of t was calculated at the level of significance $\alpha = 95\%$.

The parameters of the two tests are given in Table 4.

Table 4

Parameters of the Snedecor F *and Student* t *Tests*

	Method	
Parameter	HPLC	SP
n	8	8
X	1.000	0.999
s	0.0054	0.0075
Standard deviation	0.0019	0.0026

HPLC, high-performance liquid chromatography; SP, spectrophotometry.

DISCUSSION

The photodegradation of NV was evaluated by two methods, UV spectrophotometry and HPLC. As follows from Fig. 1, exposure of NV to light produced changes in the UV spectrum of the compound. The intensity of the band at 378 nm decreased, and new bands with the maxima at 285 and 310 nm appeared. Two isosbestic points at 272 and 328 nm were found for which the molar coefficients of absorption of NV and products of its decomposition were the same. The appearance of the band with the maximum at 285 nm is characteristic of the DHP nitroderivatives exposed to light as it is related to the reduction of the nitro to nitroso group. The decrease in the intensity of the band at about 377 nm is interpreted as corresponding to aromatization of the DHP ring.

The results of the HPLC analysis under the optimized conditions allowed a satisfactory separation of the products of NV photodecomposition. The UV detector, working at $\lambda = 377$ nm, was applied and proved the presence of two products of the photodegradation characterized by $t_R = 3.8$ min and $t_R = 4.8$ min (Fig. 2). The identity of the products was confirmed by the low- and high-resolution gas chromatography–mass spectrometry (GC-MS) mass spectra. Analysis of the processes of fragmentation revealed that the main products of NV photochemical decomposition appear as a result of oxidation of the DHP ring and elimination of a HCN molecule. Oxidation of the DHP ring leads to aromatization of the system and appearance of the so-called pyridinium derivative, that is, the 5-isopropyl-3-methyl ester of 4-(3-nitrophenyl)-6-methyl 3,5-pyridinecarboxylic acid. The product formed as a result of elimination of a HCN molecule is the 5-isopropyl-3-methyl ester of 4-(3-nitrophenyl)-6-methyl 3,5-pyridinocarboxyl acid (16).

The quantitative evaluation of the photodegradation process was performed on the basis of the quantum yields of the photochemical reactions. Their determination required measurement of the energy of radiation absorbed by a given system, which was realized by the method of chemical actinometry. The number of emitted quanta was found using Reinecke salt, which is frequently applied as a chemical actinometer.

Experimental data allowed determination of the apparent quantum yields (Table 1), and their extrapolation to zero time of irradiation gave the actual quantum yield. For NV, the actual quantum yields were on the order of 10^{-5}, which indicated the involvement of secondary photochemical reactions initiated by the primary products of decomposition. The quantum yields were much lower than unity, which is also characteristic of reactions taking place in solutions and is interpreted as a result of deactivation of excited molecules by the solvent.

The low observed quantum yield of photodecomposition of NV can be attributed to two possible factors: the occurrence of efficient secondary processes involving products of primary photochemical reaction resulting in formation of the chemically unchanged substrates in the ground state and the high rates of radiationless deactivation processes of the reactive excited state of NV. The importance of the latter factor is confirmed by the low value of fluorescence quantum yield of NV.

In the following, a quantitative evaluation of the process of photodegradation was made through determination of the kinetic parameters, assuming the first-order reaction. From the results obtained by the spectrophotometric method and HPLC, the rate constants of the reaction k, time of decomposition of 50% of the substance $t_{0.5}$, and time of decomposition of 10% of the substance $t_{0.1}$ were determined.

The analytical methods applied enabled the calculation of the kinetic parameters of NV photodegradation, considered as a function $\ln c = f(t)$.

The methods applied to evaluate the photodegradation of NV were subjected to a procedure of validation to check if the two methods can be useful for the quantitative assessment of the photodegradation. The validation procedure included a determination of sensitivity, range of linearity, precision, limit of detectability and determinability, and the specificity of the methods. At the first stage of the validation procedure, the sensitivity and range of linearity of the methods were established. The calibration curves revealed a linear dependence of the absorbance or peak area on the NV concentration. On the basis of the verification of the hypothesis of significance of the b coefficient, it was found that, in all cases, the real value of b tends to zero, and the calibration curves can be described by the equation $y = ax$. As indicated by the confidence interval of the parameters of regression b, the validated methods are not charged with a constant systematic error since the intervals cover zero.

The sensitivity of the methods can be inferred from the slope of the curve $y = f(x)$. A comparison of the slope of the regression lines shows that the HPLC method is characterized by greater sensitivity. Precision of the two methods was evaluated on the basis of the often-repeated determinations of NV in a solution of its known concentration. The following statistical parameters characterizing the scatter of results (Table 3) indicate the greater precision of the HPLC method: the standard deviation of individual result S, relative standard deviation S_y, and confidence intervals. The validation procedure also requires determination of the specificity of the methods, as well as the limits of detectability and determinability.

Of the two methods, only HPLC can be considered specific since its optimization allowed simultaneous determination of nondecomposed NV in the presence of the products of its decomposition (Fig. 2).

Statistical comparison of the two methods was also made. By the Snedecor F test, the zero hypothesis of the identity of variances determined by the two methods at a certain level of significance was verified. The calculated value of F was compared with the critical value $F\alpha_{(n1\text{-}1,\ n2\text{-}1)}$ to find $F < F_{\alpha(n1-1,\, n2-1)}$. The result testifies to a statistically insignificant difference between the variances of the two methods, therefore indicating their good precision. The hypothesis on the identity of the means of the results obtained by the two methods was verified by the Student t test. The value of t was calculated at the assumed level of significance for $n_1 = n_2$ and compared with the critical value $t_{\alpha,f}$ from the tables. It was found that $t_{\alpha,f} > t$, which proves that the difference between the means provided by the two methods is statistically insignificant.

CONCLUSION

Radiationless processes are mainly responsible for deactivation of the excited states of NV. Long exposure to UV radiation led to photochemical changes, resulting in loss of the pharmaceutical properties of the compound studied.

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