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Identification of photodegradation products of nilvadipine using GC-MS

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Abstract

Nilvadipine (NV) photodegradation products have been analysed with gas chromatography-mass spectrometry (GC-MS). The 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 UV lamp type — HBO 200. The maximum intensity at the wavelength $\lambda = 365$ nm was achieved by applying the interference filter and Wood's filter. Using the Reinecke salt as a chemical actinometer, apparent quantum yields of photodegradation were obtained, which after extrapolation to the zero time of irradiation gave the actual quantum yield ($\varphi = 7.58 \times 10^{-5}$). The structure of three nilvadipine photodegradation products was established, after mass spectra analysis of compounds registered during GC-MS carried out of irradiated nilvadipine solutions. The quantitative results of GC-MS analyses enabled to determination of the kinetic parameters of NV photodegradation, calculated from the dependence $\ln c = f(t)$. Quantitatively the process was described with the calculated rate constant of decomposition (k), decomposition time of 50% of the compound ($t_{0.5}$) and decomposition time of 10% of the compound ($t_{0.1}$). The exposure of nilvadipine to UV light was found to lead to aromatization of the DHP ring and elimination of the HCN molecule.

Keywords: Nilvadipine; 1.4-Dihydropyridine derivatives; Photodegradation; Mass spectrum

1. Introduction

Nilvadipine (NV) is a calcium channel blocker very useful in hypertension therapy, although vasodilatation and reflex tachycardia often represent undesired effects [1–6].

It is known that calcium antagonists from the group of 1,4-dihydropyridine derivatives are pho-

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tolabile and the products of their photochemical decomposition have no pharmacological activity [7–12]. Like the other DHP derivatives, after exposure to light, NV undergoes decomposition leading to a weakening or cessation of the desired pharmacological activity. In general, the information on the drugs photoreactivity is probably not sufficient among practitioners of pharmaceutical chemistry. Reports on this subject have been growing in number in the recent years, but they are scattered in a variety of journals, thus possibly not reaching all interested readers. Although testing the photostability of pharmaceuticals is not a new idea, development of a standard procedure for such tests arouses many controversies. In particular of much need is a development of an appropriate photodegradation test, which would directly answer the question whether a given substance is stable in a given model of exposure to light. Although there are numerous reports on photostability of pharmaceutical drug substances and drug products in literature, few articles have addressed procedures and protocols for performance of such studies [13,14]. For many years efforts have been made to find a test for photostability which would establish in a simple way whether a given drug or therapeutic substance is stable in the assumed model of exposure to light. The International Conference on Harmonization (ICH), Expert Working Group (EWG) for photostability testing has proposed guidelines for photostability testing. According to the ICH document two procedures for determination of photostability have been recommended. In the studies on photodegradation of drugs it was suggested to take into account the effect due to illumination of rooms, and to eliminate the influence of the high-energy ultraviolet radiation [15]. Regarding the increasing use of DHP derivatives in medical treatment, not only in cardiology, the studies on their photochemical stability are still of considerable interest.

The aim of our studies reported in this work was identification of certain photodegradation products of NV with gas chromatography mass spectrometry and estimation of yields of nilvadipine photodegradation.

2. Experimental

2.1. Materials

Nilvadipine: 5-isopropyl 3-methyl 2-cyano-4-(3-nitrophenyl)-6-methyl-1,4-dihydro-3,5-pyridinedicarboxylate — $C_{19}H_{19}N_3O_6$ (AN 1546), was obtained from Klinge Pharma. Solvents: acetonitryl; methanol; hexane of HPLC grade were purchased from Baker.

2.2. Irradiation conditions

NV methanol solution of the concentration 1.9×10^{-4} mol/l was placed in a cylinder, quartz cell of 2.4 ml capacity and $l = 1$ cm in length, and then irradiated from a source at a distance of 30 cm. The irradiation was performed with the high-pressure UV lamp equipped with a mercury burner HBO-200 and the maximum intensity of the radiation of $\lambda = 365$ nm was obtained using the interference and Wood's filters.

The solutions studied were irradiated for 1, 3, 5, 7 and 10 h, and then concentrated in a stream of nitrogen. Dried residue was dissolved in 10 μl methanol.

GC chromatograms were obtained using a solution of felodipine (FL), of the concentration of 1.24×10^{-2} mol/l as internal standard. Each time a portion of 1 μl the analysed NV solution and FL was deposited on the column.

2.3. Instrumentation

2.3.1. UV measurements

Analyses were performed on spectrophotometer Jasco, type V-550.

2.3.2. Direct introduction electron impact ionization mass spectrometry (EI MS)

Low and high resolution EI mass spectra were registered on AMD Intectra (Germany) double focusing, reversed geometry B/E mass spectrometer Model 402 operating at ionization voltage of 70 V and source temperature 200°C, samples were evaporated from heated metal capillary and introduced to the source of mass spectrometer.

2.3.3. Analysis of mixtures with gas chromatography-mass spectrometry

Samples were subjected to GC/MS analyses on a Hewlett-Packard Gas Chromatograph model 5890II with Mass Selective Detector model 5971A. The instrument was equipped with DB-5 (J&W, USA) fused silica capillary column (30 m × 0.25 mm ID). The carrier gas was helium at a flow rate 1 ml/min. The column temperature was programmed from 140°C (held for 2 min) at rate 5°C/min to 300°C, which was maintained for 5 min. The injector temperature was 250°C. Mass spectra were recorded in the range of 50–650 u.

3. Results and discussion

3.1. Quantum yield of NV photodegradation products

The quantum yield of NV photodegradation was determined using the Reinecke salt as a chemical actinometer. The NV solutions were irradiated until about 60% conversion determined from a difference in the absorbance measured before and after a given irradiation time ($\lambda = 377.6$ nm; $l = 1$ cm).

The quantum yield for a given percent of conversion was calculated from the formula:

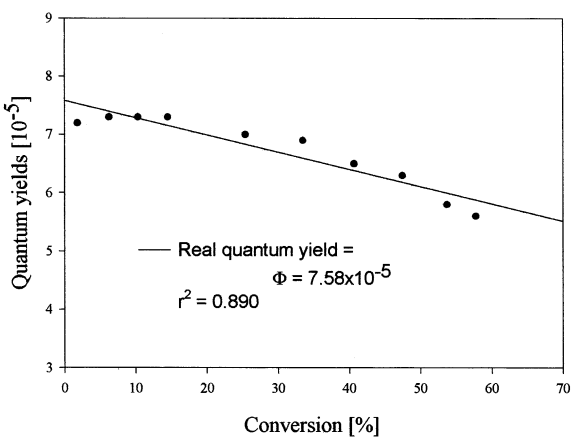

Fig. 1. Quantum yields of the nilvadipine photodegradation ($\lambda_{exe} = 365$ nm).

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

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, t is time (s) [16].

The obtained dependence of the percent of NV conversion on the time of irradiation and the quantum yield are given in Fig. 1. In order to get real quantum yields, the yields established in experiment for different times of conversion (percent conversion) were extrapolated to the initial intensity of NV (0% of conversion).

Experimentally determined quantum yields for particular irradiation times were extrapolated to the initial NV concentration to obtain the real quantum yield of $\phi = 7.58 \times 10^{-5}$, which suggests the occurrence of secondary photochemical reactions initiated by the primary products of decomposition — Fig. 1.

3.2. Identification of photodegradation products

Photodegradation products obtained during irradiation of nilvadipine with UV light were identified on the basis of registered mass spectra. This instrumental method was applied earlier for recognition of photodegradation products of other calcium channel blockers of the DHP derivatives [17–21]. Fragmentation pathway of nilvadipine was established from low and high resolution MS spectra recorded after direct introduction of sample to AMD mass spectrometer (Fig. 2 and Table 1). In the molecular ion $M^{+\bullet}$ of nilvadipine cleavages of bonds in isopropyl ester group attached to the DHP ring was observed. There occurred elimination of isopropyl $[M-{}^{\bullet}C_3H_7]^+$ or isopropyloxy $[M-{}^{\bullet}OC_3H_7]^+$ radicals, m/z 342 and 326, respectively. Rupture of the bond between DHP ring and carbon atom of ester group $[M-{}^{\bullet}COOC_3H_7]^+$ group, ion at m/z 298 was also registered. Fragment ions created after elimination of hydroxyl $[M-{}^{\bullet}OH]^+$ at m/z 368 were observed. The main ion in the mass spectrum was fragment created after cleavage of bond between phenyl and DHP ring with the retention of the charge on the last mentioned ring m/z 221.

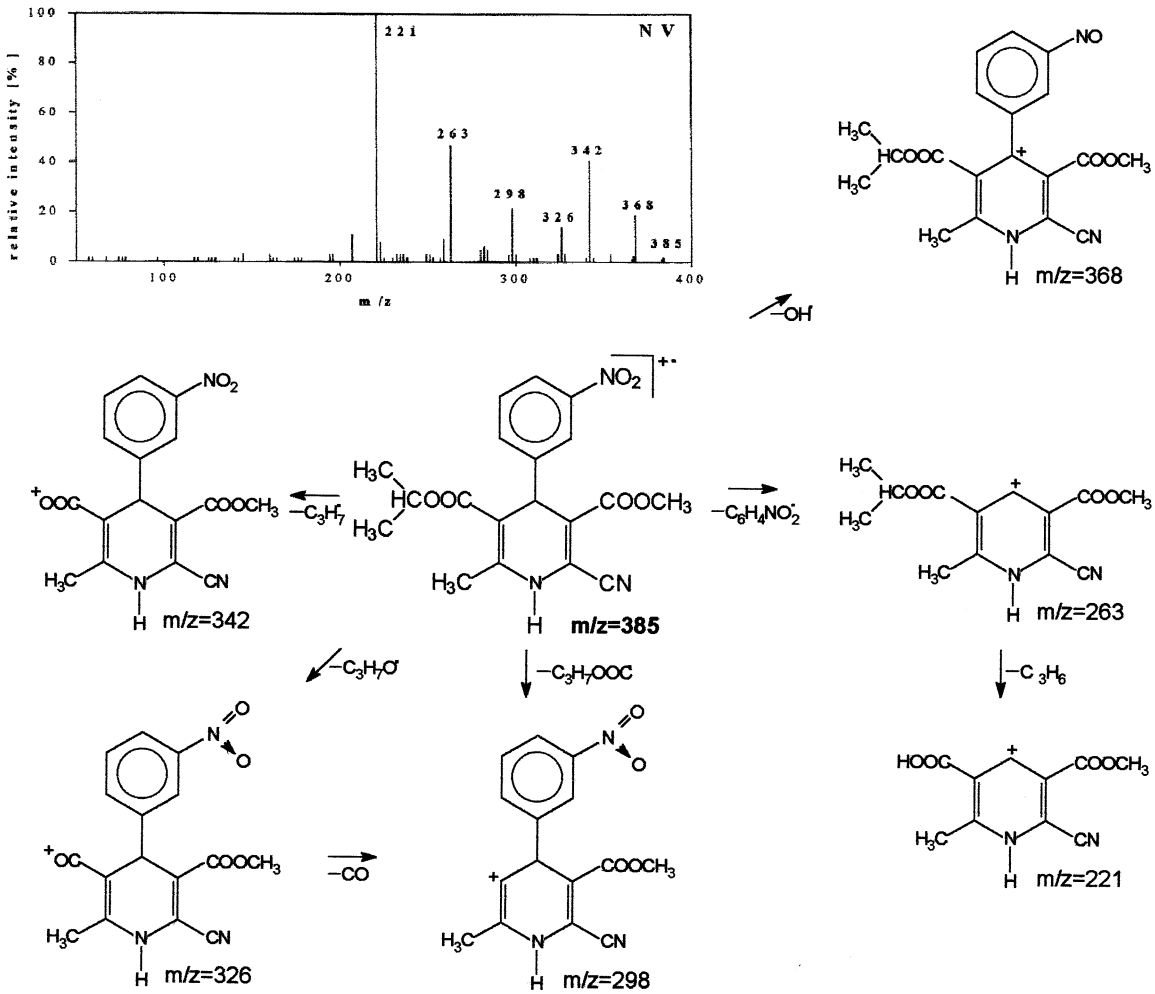

Fig. 2. Mass spectrum and fragmentation pathway of nilvadipine.

Structures of nilvadipine photoproducts (**I**–**III**) created during irradiation with UV light were elucidated from GC/MS electron impact mass spectra registered for samples of the irradiated mixtures of NV. On the total ions current chromatograms (TIC) of the samples obtained after four different times of irradiation were present peaks of five photoproducts (Fig. 3). The structures of three compounds were established on the basis of molecular masses and fragmentation pathways evaluated from the mass spectra (Fig. 4). Compound (**I**) eluting from the column at RT = 17.13 min was noticed only in the mixture irradiated for 180 min. The second photoproduct (**II**), RT = 19.73 min was present in all studied samples obtained after exposure to UV light. Compound (**III**) eluted at RT = 21.50 min was recorded in two samples with extended irradiation times (420 and 600 min). In two mixtures with the highest time of irradiation two other peaks of photoproducts (**IV**) and (**V**) were registered at RT = 21.88 min (420 min) and RT = 23.85 min (600 min), respectively. The structures of these last two mentioned photoproducts were not established — only their molecular masses were determined at $m/z = 356$ and 316, respectively.

Photoproduct (**I**) was recognised as 3-methyl 5-isopropyl 2-cyano-4-(3-nitrophenyl)-6-methyl-3,5-dicarboxylate, molecular mass 383 Da. This compound was created due to oxidation of DHP ring to pyridine in nilvadipine molecule. The mass difference between the both compounds (**I** and NV) indicated a creation of additional double bond in the molecule of this photoproduct. The fragmentation of photoproduct (**I**) is much reacher than of intact nilvadipine. In the mass spectrum fragment ions created after elimination from molecular ion both alkyl substituents (methyl or isopropyl) present in ester groups, attached to pyridine ring were observed (Fig. 5). Rupture of the bond between both rings pyridine and benzene, as it was observed in nilvadipine mass spectrum did not occurred. Aromatization of dihydropyridine ring, recognised in all identified photoproducts, might inhibit this way of fragmentation.

In the mass spectrum of the second compound (**II**) (RT = 17.13 min), molecular ion $M^{+\bullet}$ was registered at m/z 358. Even molecular mass of this compound and mass difference with the molecular mass of nilvadipine, testify about loss of cyano group and aromatization of DHP ring to pyridine. The main fragmentation pathway of this compound was based on the alternative elimination of one of two ester substituents. In the mass spectrum fragments created after rupture of bonds in both esterifical carboxyl group, in the first

Table 1
The elemental composition of the nilvadipine fragment ions

Obtained mass	Calculated mass	Formula
221.0571	221.0562	$C_{10}H_9N_2O_4$
263.1034	263.1032	$C_{13}H_{15}N_2O_4$
282.0749	282.0754	$C_{14}H_{10}N_3O_4$
284.0684	284.0671	$C_{14}H_{10}N_3O_4$
298.0821	298.0828	$C_{15}H_{12}N_3O_4$
326.0749	326.0777	$C_{15}H_{12}N_3O_4$
342.0743	342.0726	$C_{16}H_{12}N_3O_6$
368.1261	368.1246	$C_{19}H_{18}N_3O_5$
385.1252	385.1274	$C_{19}H_{19}N_3O_6$

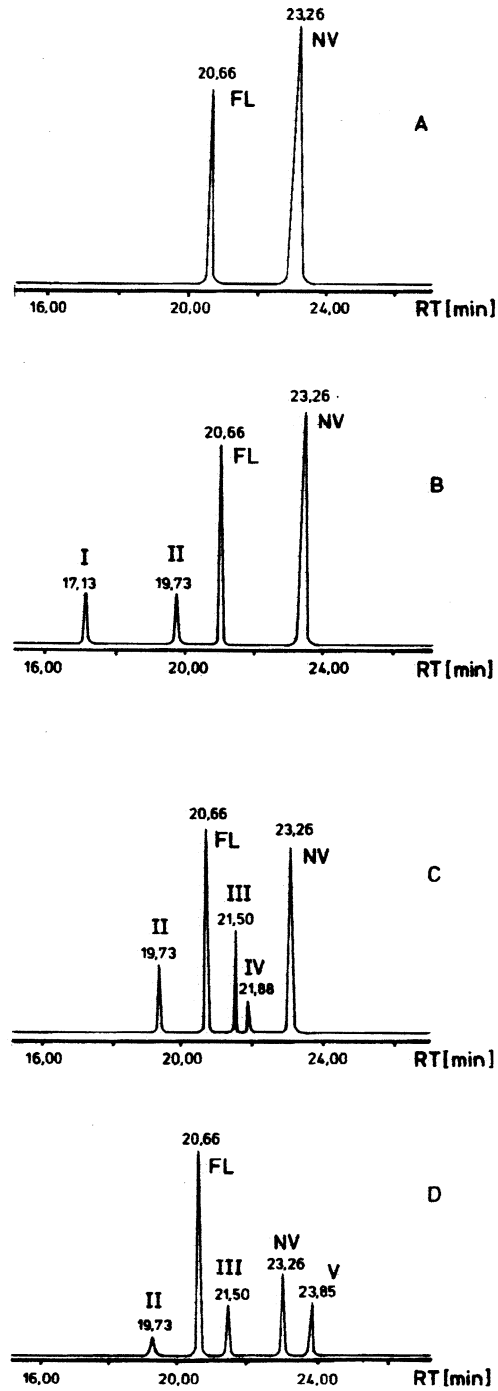

Fig. 3. The total ion current chromatograms registered for samples of nilvadipine after different time of UV irradiation. Irradiation time: A = 0 min; B = 180 min; C = 420 min; D = 600 min

fragmentation step, both ions at $m/z = 249$ and 316 were observed (Fig. 6). The characteristic feature of fragmentation of photoproduct (**II**) was creation of ions with condensed third, five-member ring. These ions were created after elimination from different fragments neutral molecules (alcohol or water). There was also observed elimination of HNO_2 (nitrous acid) molecule in the third fragmentation step.

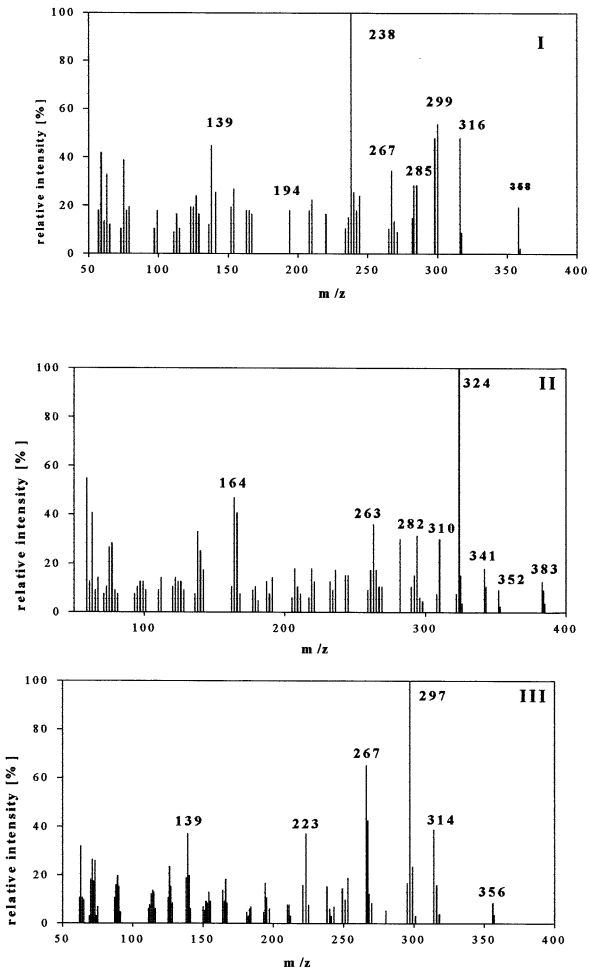

Fig. 4. Mass spectra of the photodegradation products of nilvadipine. (**I**) 5-isopropyl 3-methyl 6-methyl-4-(3-nitrophenyl) 3,5-pyridinedicarboxylate. (**II**) 5-isopropyl 3-methyl 2-cyano-6-methyl-4-(3-nitrophenyl) 3,5-pyridine- dicarboxylate. (**III**) 3-isopropyl 2-methyl-5-oxo-7H-furo-[3,4-b]-3-pyridinecarboxylate.

The last identified photoproduct (**III**) (RT = 21.50 min) has molecular mass 356 Da. In the molecule of this photoproduct was created the third furan ring based on the cyclization of methyl ester on the pyridine moiety obtained after aromatization. The fragmentation pathway of compound (**III**) is presented in Fig. 7. The involvement of methyl ester group in rearrangement of nilvadipine is supported with lack of fragment ion created after elimination of methoxyl radical or neutral methanol molecule. This fragmentation pathway of molecular ion in the mass spectra of earlier recognised photoproducts (**I**) and (**II**) was observed. The main ion in the mass spectrum at $m/z = 297$ after cleavage from molecular ion of isopropyloxy radical was created. There were also observed ion at m/z 314 due to elimination of propene neutral molecule from M^+. Further elimination of nitrous acid was also registered.

3.3. Quantitative description of the photochemical process

The calibration curve of NV was determined for the concentrations from 1.0×10^{-2} to 2.0×10^{-3} mol/l. The internal standard was a solution of felodipine (FL) in methanol of the concentration of 1.2×10^{-2} mol/l. Each time 1 μl of NV solution and 1 μl of the internal standard were deposited on the column. In the calculations the ratios of the heights of the peaks due to the substance studied and the internal standard were taken into regard.

In the conditions studied, a linear dependence was obtained between the slopes of the lines $h_i/h_s = f(c)$ for NV concentration ($r^2 = 0.974$).

The photochemical decomposition of NV was determined by GC-MS method according to the equation:

$$\ln h_i/h_s = \ln h_0/h_s - k \cdot t$$

where: h_i and h_0 are the heights of the peaks of NV at the time $t = 0$ and $t =$ specific time after irradiation; h_s is the height of the peak of the internal standard (FL); k is the rate constant.

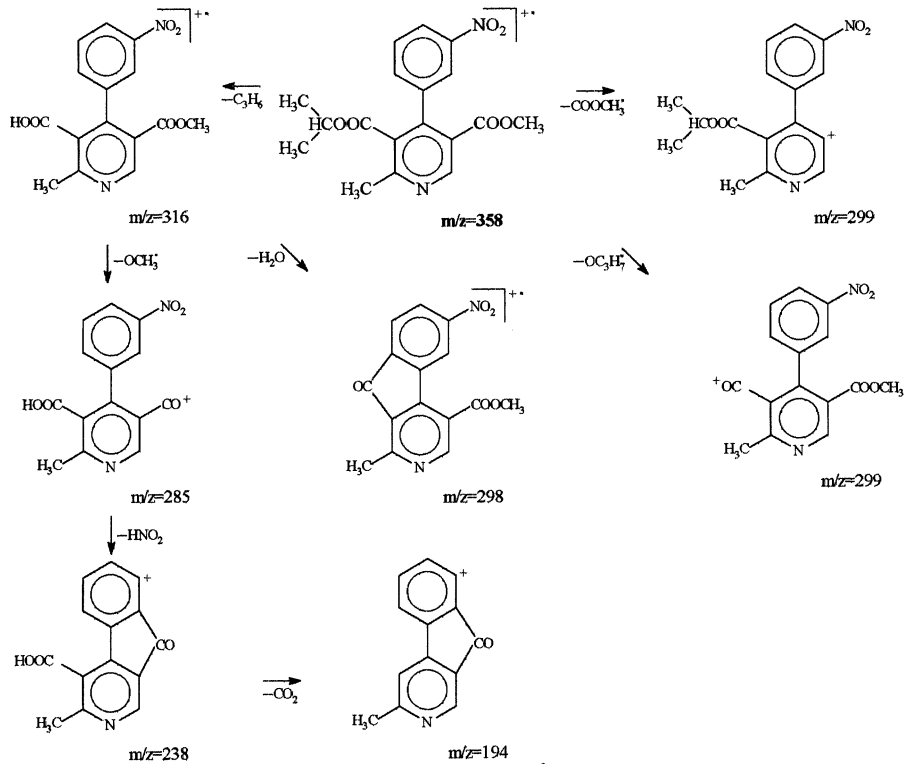

Fig. 5. Scheme of the fragmentation pathway of 5-isopropyl 3-methyl 6-methyl-4-(3-nitrophenyl) 3,5-pyridinedicarboxylate (**I**).

The observed rate constant (k) was equal to the slope of plot of $\ln h_i/h_s = f(t)$, taken with the opposite sign.

The results were used for quantitative assessment of the photodegradation described by the parameters:

rate constant of the first order reaction: $k_{obs} = 1.34 \times 10^{-3} \pm 7.0 \times 10^{-5}$ (min^{-1})

time of half decomposition: $t_{0.5} = 518.1 \pm 26.3$ (min)

time of decomposition of 10% of the compound: $t_{0.1} = 78.8 \pm 4.0$ (min).

The each experiment was performed three times.

4. Conclusions

1. The quantum yield of photochemical decomposition of NV, equal 7.58×10^{-5}, suggests the occurrence of secondary photochemical processes initiated by the primary products of NV decomposition.
2. In the first step of nilvadipine photodegradation under UV light aromatization of DHP ring occurred, and further rearrangement was connected with elimination of substituents present on heteroaromatic ring.
3. The main products of nilvadipine photodegradation are:

   (**I**) 5-isopropyl 3-methyl 4-(3-nitrophenyl)-6-methyl-3,5-pyridinedicarboxylate

   (**II**) 5-isopropyl 3-methyl 2-cyano-4-(3-nitrophenyl)-6-methyl-3,5-pyridinedicarboxy--late

   (**III**) 3-isopropyl 2-methyl 4-(3-nitrophenyl)-5-oxo-7H-furo-[3,4-b]-pyridine-3-carboxylate.

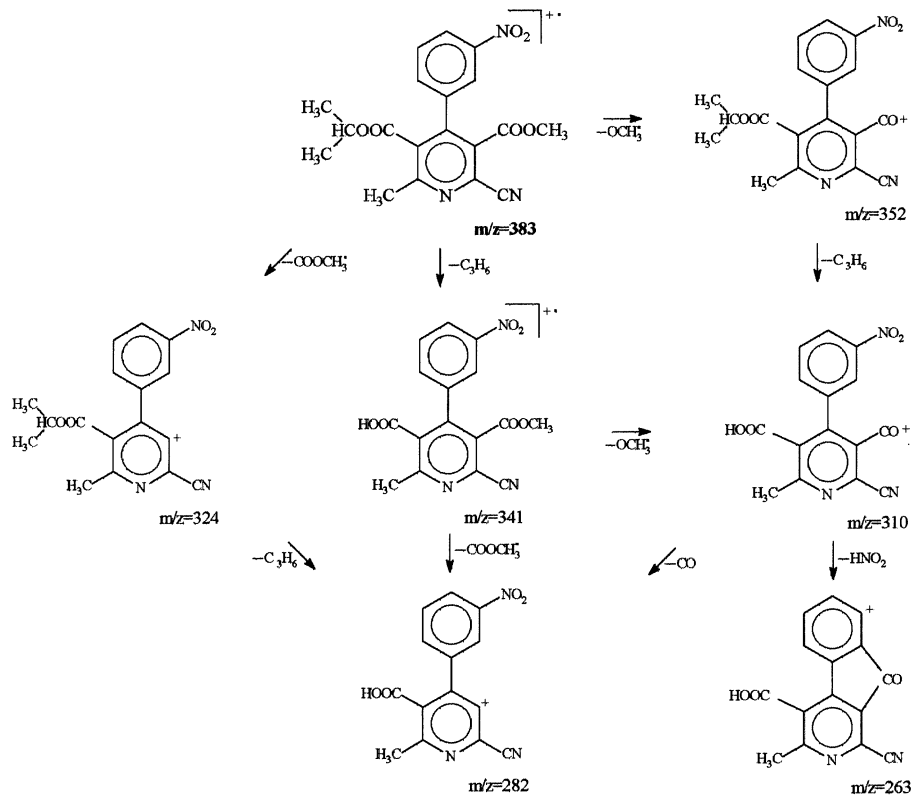

Fig. 6. Scheme of the fragmentation pathway of 5-isopropyl 3-methyl 2-cyano-6-methyl-4-(3-nitrophenyl) 3,5-pyridinedicarboxylate (**II**).

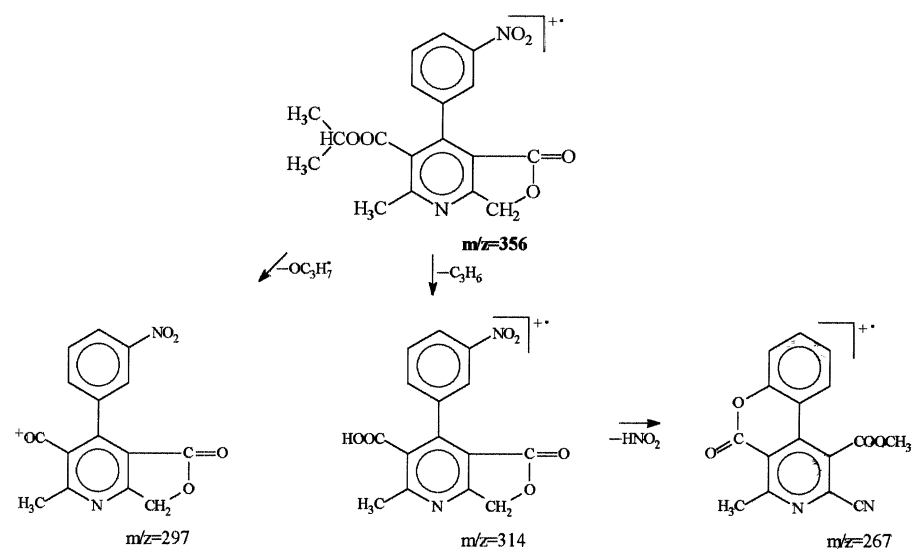

Fig. 7. Scheme of the fragmentation pathway of 3-isopropyl 2-methyl-5-oxo-7H-furo- [3,4-b]-3-pyridinecarboxylate (**III**).

Acknowledgements

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