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Inclusion Complexes of Manidipine with γ-Cyclodextrin and Identification of Photodegradation Products

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Abstract

The paper presents results of a study of photochemical decomposition of manidipine and its inclusion complexes with γ-cyclodextrin and identification of photodegradation products. The process was qualitatively assessed by the UV spectrophotometry and by HPLC-MS. The quantitative assessment of its efficiency was performed on the basis of kinetic parameters and quantum yields. The main product of photodegradation was nitrophenylpyridine derivative, while the concentration of nitrozophenylpyridine derivative being the other product of this process, was about 20 times lower. The inclusion complexes of manidipine with γ-cyclodextrin were obtained in the liquid phase. The stoichiometry of the complexes was determined from the Benesi–Hildebrand equation. The photochemical stability of manidipine in inclusion complexes was compared with that of manidipine in non-complexed form.

Introduction

Manidipine is a dihydropyridine calcium channel blocker (DHP) with a piperazinyl group, used in the treatment of hypertension. In general, the reduction in blood pressure caused by manidipine is similar to that observed as an effect of administration of amlodipine or nifedipine, but with a peculiar pharmacokinetic profile [1, 2].

Manidipine causes systemic vasodilation by inhibiting the voltage-dependent calcium inward currents in smooth muscle cells [3, 4]. The resulting reduction in blood pressure in patients with hypertension is maintained over 24 h [5–7].

The drug needs to be administered only once a day and is used for the treatment of mild-to-moderate essential hypertension, of hypertension associated with renal impairment or diabetes mellitus, and severe hypertension [8–11]. In healthy human volunteers, absorption of an oral dose of manidipine was proved rapid, and peak plasma concentration was reached after 1–2 h, declining biphasically with an apparent elimination half-life of 4–8 h. Manidipine is extensively metabolised in the liver, through de-esterification, cleavage of diphenylmethylpiperazinyl or diphenylmethyl groups, and amortisation of the dihydropyridine moiety [12].

From the point of view of the use in treatment, DHP derivatives reveal some undesirable characteristics such as high photochemical sensitivity, which lead to chemical changes responsible for weakening of the therapeutic effect [13–15]. One of the methods of upgrading the physical and chemical properties of DHP derivatives is based on preparation of inclusion complexes of these drugs with cyclodextrins [16, 17].

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 [18–26].

Experimental

Material and apparatus

Manidipine (MN) 2-[4-(diphenylmethyl)-1-piperazinyl]ethylmethyl(±)-1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate ($C_{35}H_{38}N_4O_6 \cdot 2HCl$) was received from dr Gianni Motta (Industria Chemica et Farmaceutica S. P. A., Milano, Ytaly). MW 683.65 g/mol.

Liquid chromatograph, model 2690 (Waters) with UV-Photodiode Array (Waters) and Millennium 2000 software.

Mass spectrometer ZQ (Waters/Micromass).

Photodegradation process of manidipine

Photodegradation studies

The photodegradation process was run following version 1 of ICH recommendation. The methanol solution of manidipine of the concentration 6.43×10^{-5} mol/dm^3

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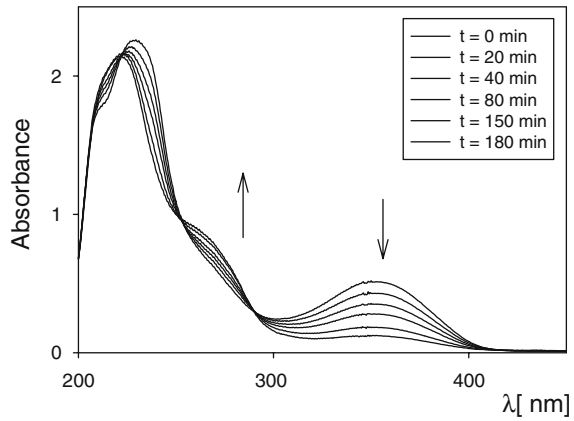

Figure 1. UV spectra of manidipine after different times of photodegradaftion (0–180 min).

was irradiated in a cylindrical quartz cell (volume = 2.8 cm^3, l = 1 cm) with a high-pressure UV HBO-50 lamp with a mercury burner, emitting radiation in the range 300–400 nm. The maximum absorption at 365 was obtained using a CC-4 filter.

After irradiation for certain periods (from 0 to 180 min), the UV spectra were made in the range 200–400 nm, see Figure 1.

The photochemical decomposition of manidipine was also studied by HPLC, using an RP-18 LiChrospher (4 × 250 mm) column, made by Merck. The mobile phase was a mixture of a phosphate buffer (pH 4.5):acetonitrile = 9:1 (v/v); the flow rate of the mobile phase through the column was 0.7 ml/min.

The chromatogram and UV spectra of manidipine and its photodegradation products are shown in Figure 2.

Identification of the photoproducts of manidipine – HPLC-MS method

The manidipine solution of the concentration 6.45×10^{-5} mol/dm^3 was subjected to irradiation for 20, 40, 60 and 180 min, and then analysed by the HPLC-MS method. The conditions: C-18 Novapack (3.9 × 150 mm) column, made by Waters; the mobile phase flow rate 0.5 ml/min.

The products of the photodegradation were separated by gradient elution. At the beginning the mobile phase was a mixture of methanol:water:10% formic acid at the ratio 20:75:5 (v/v/v). During the analysis the contribution of methanol was increased so that 20 min after the beginning of the analysis the mobile phase was 100% methanol. The mass spectra of manidipine and its separated photoproducts were recorded in the range 100–1000 [M-zH^+], using the Electron Spray Ionization (ESI) method. Molecular ions were obtained at [M-zH^+] = 611 manidipine, 609 – nitrophenylpyridine derivative and 595 – nitrozophenylpyridine derivative. Mass spectra (LC-ESI-MS) of manidipine and products of its photodegradation are shown in Figure 3.

Validation of the analytical methods

The suitability of the analytical methods applied was verified by determination of the methods' linearity, range and precision.

The linear regression analysis of manidipine was made by plotting the peak area (y) and the absorbance versus MN concentration (x) in mol/dm^3. The calibration curves were drawn in the range – 6.50×10^{-5} – 9.28×10^{-6} mol/dm^3.

The following equations were obtained:

HPLC

$$y = 1.03 \times 10^{10}x + 15328.6 \quad r = 0.998$$

SP – spectrophotometric method

$$y = 335.08x + 1.51 \times 10^{-4} \quad r = 0.998$$

Precision. The parameters characterising the precision of the HPLC and spectrophotometric method are given in Table 1.

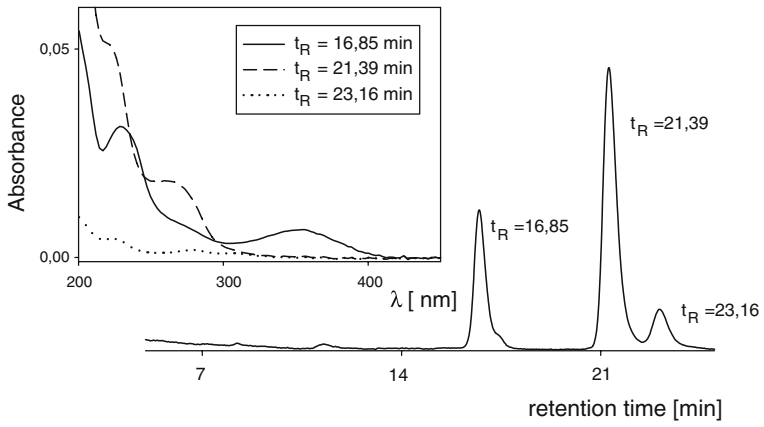

Figure 2. HPLC chromatogram and UV spectra of manidipine and its photodegradation products after 180 min photodegradation: Retention time: t_R = 16.84 min – manidipine; t_R = 21.39 min major degradation product I – nitrophenylpyridine derivative; t_R = 23.16 min minor degradation product II – nitrozophenylpyridine derivative.

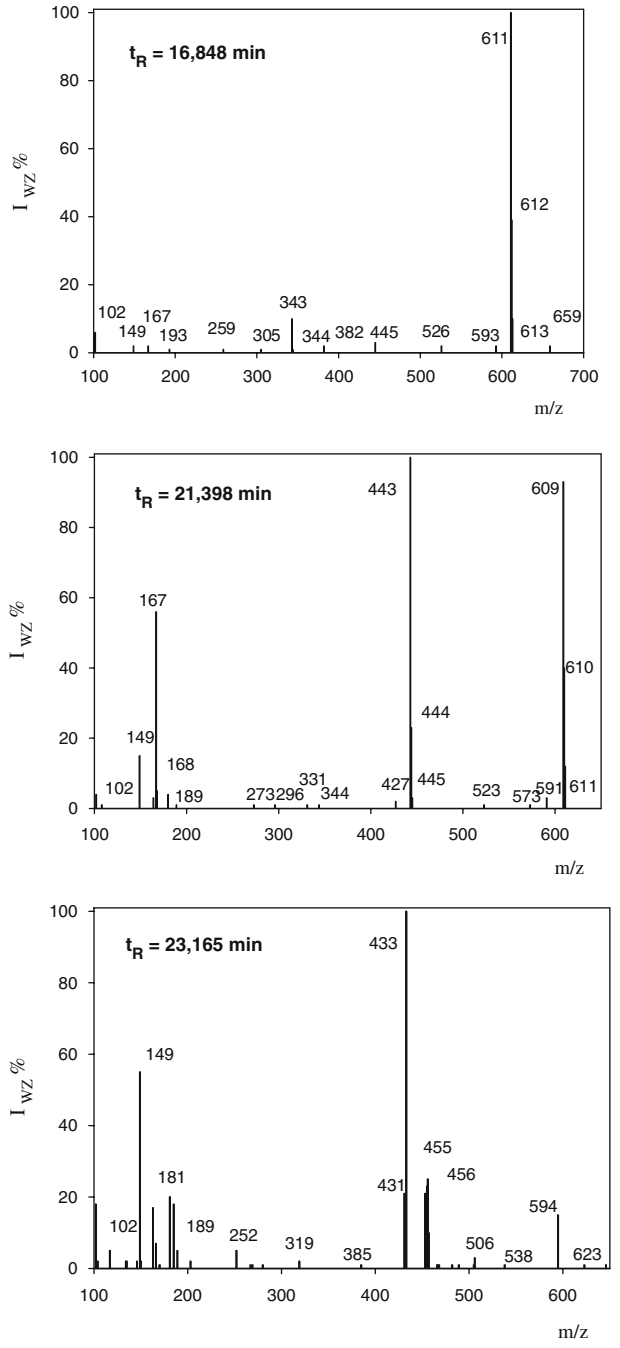

Figure 3. Mass spectra (LC-ESI-MS) of manidipine and products of its photodegradation (1) manidipine ($t_R = 16.848$ min); (2) photoproduct I ($t_R = 21.398$ min) – nitrophenylpyridine derivative of manidipine; (3) photoproduct II ($t_R = 23.165$ min) – nitrozophenylpyridine derivative of manidipine.

Quantitative studies

The kinetic parameters. The results concerning the manidipine photodegradation obtained by UV spectrometry and HPLC permitted determination of the kinetic parameters of the process described by the dependence $\ln c = f(t)$. The values of the reaction rate constant (k), half-life time ($t_{0,5}$) and time of decomposition of 10% of the compound ($t_{0,1}$) were determined, see Table 2.

Table 1. Validation of the spectrophotometric and HPLC method for the determination of manidipine precision

Method	HPLC	SP
n	8	8
X	3.45×10^{-5}	3.60×10^{-5}
S^2	3.14×10^{-14}	2.08×10^{-14}
S	1.77×10^{-7}	1.44×10^{-7}
S_y	5.13×10^{-3}	5.42×10^{-3}
S_x	1.33×10^{-7}	1.06×10^{-7}

Quantum yield. Quantitative assessment of the process of photodegradation was performed on the basis of quantum yields of the photochemical reactions involved. The quantum yields were determined with the use of the Reinecke salt as a chemical actinometer. The experimental quantum yields corresponding to particular irradiation times were extrapolated to the initial concentration of manidipine in order to obtain the actual quantum yield. The obtained values of quantum yield of photodegradation, determined by the methods HPLC and SP, were $\varphi = 3.443 \times 10^{-5}$ and $\varphi = 3.244 \times 10^{-5}$.

Inclusion complexes of manidipine with γ-cyclodextrin (γ-CD)

Preparation of the inclusion complexes

To aqueous solution of manidipine of the concentration 5.46×10^{-5} mol/dm^3 placed in a quartz cell of 3 cm^3 in capacity, γ-CD was added in portions (from 0.11 mg to 1.04 mg), to obtain samples of its concentration in the range 2.83×10^{-5} – 2.67×10^{-4} mol γ-CD/dm^3. After addition of each subsequent portion of γ-CD, the sample was stirred by a magnetic stirred for 15 min, and then the UV spectra of the samples were taken in the range 200–450 nm and the samples' absorbency was measured ($\lambda = 356$ nm).

Stoichiometry of the complexes

The stoichiometry of manidipine complexes was determined by the Benesi-Hildebrand equation. This equation applied to describe changes in the absorbency as a parameter measured takes the following linear form:

$$A - A_0 = \frac{1}{K(\varepsilon_{G\cdot\mathrm{CD}} - \varepsilon_G)[G] \cdot l \cdot [\mathrm{CD}]_T} + \frac{1}{(\varepsilon_{G\cdot\mathrm{CD}} - \varepsilon_G)[G]_T \cdot l} \quad (1)$$

Table 2. Kinetic parameters of photochemical decomposition of manidipine

Method	k [min$^{-1}10^3$]	$t_{0,5}$ [min]	$t_{0,1}$ [min]
SP	9.05	76.58	11.64
HPLC	8.94	77.58	11.79

A_0 – absorbance of manidipine in non-complexed form; A – absorbance of manidipine in inclusion complexes; $\varepsilon_{G\cdot CD}$ i ε_G – molar coefficient of absorbance of the manidipine in inclusion complexes and in non-complexed form; l – length of the optical way (l = 1 cm); $[G]_T$ – concentration of the guest (manidipine); $[CD]_T$ – concentration of γ-cyclodextrin; K – the stability constant of the complex.

The differences between the initial absorbency of manidipine (A_0) and the absorbency of its solution after addition of subsequent portions of γ-CD (A) are shown in Figure 4, as a dependence of A_0-A on the concentration of γ-CD.

The stability constants of the complexes were found from the fit of the function describing the absorbency changes due to complex formation of manidipine and γ-CD to the experimental points. The dependence of $(A_0-A)^{-1}$ on $[\gamma\text{-CD}]^{-1}$ was linear, which confirmed the formation of 1:1 complex, Figure 3.

The parameters of Benesi-Hildebrand equation were calculated from the formulae:

the slope (a) of the line $A_0-A = f(\gamma\text{-CD})$

$$a = \frac{1}{K \cdot \Delta\varepsilon \cdot [c]} = 9.7 \cdot 10^{-4}$$

the point of intersection (b) of the axis y and the line describing the function: A_0-$A = f(\gamma\text{-CD})$

$$b = \frac{1}{\Delta\varepsilon \cdot [c]} = 32.91$$

the difference in the molar absorption coefficients (ε) between the complexed and non-complexed phase

$$\Delta\varepsilon = \frac{1}{b \cdot [c]} = 5.56 \times 10^2 \mathrm{dm^3 mol^{-1}}$$

stability constants of the complex (K)

$$K = \frac{1}{a \cdot \Delta\varepsilon \cdot [c]} = 3.39 \times 10^4 [\mathrm{M}]^{-1}$$

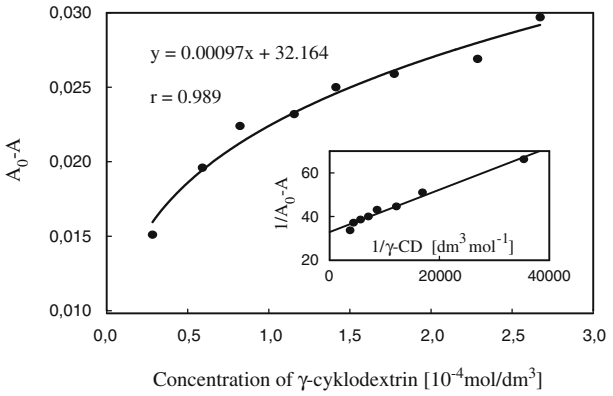

Figure 4. Benesi-Hildebrand plot for manidipine in solution of γ-cyclodextrin.

Photochemical stability of the inclusion complexes

The inclusion complexes of the γ-CD concentrations 4.52×10^{-5} and 2.67×10^{-4} mol/dm^3, prepared according to the procedure described in Preparation of the inclusion complexes, were subjected to the process of photodegradation. The process of photodegradation was conducted as in Photodegradation studies. The kinetic parameters of photodegradation of manidipine in the inclusion complexes were calculated assuming it to be a first order reaction:

$$k = 6.92 \times 10^{-3}\mathrm{min^{-1}} \quad t_{0.5} = 100.1 \text{ min} \quad t_{0.1} = 20 \text{ min}.$$

Results and discussion

The processes of manidipine photodegradation was performed following version 1 of the ICH document currently in force in the study of photostability of drugs and therapeutic substances. The methanol solution of manidipine was irradiated with a high-pressure UV lamp with the maximum emission at $\lambda = 365$ nm, close to the analytical wavelength of the compound studied. The required irradiation conditions were ensured by the use of an appropriate filter.

A Reinecke salt solution was irradiated with the wavelength $\lambda = 365$ nm for 60 s. The number of quanta absorbed by the actinometer was 2.69×10^{16} and was equal to the number of quanta falling on the manidipine solution studied in the time of 60 s. The energy of a quantum of radiation of $\lambda = 365$ nm was calculated from the formula

$$E = h \cdot \frac{c}{\lambda} = 6.624 \times 10^{-34}\mathrm{J{\cdot}s}\frac{2.998 \cdot 10^8 \mathrm{ms^{-1}}}{365 \cdot 10^{-9}\mathrm{m}} = 5.441 \times 10^{-19}\mathrm{J}.$$

The intensity of irradiation (P) absorbed by the actinometer was

$$P = \frac{5.441 \times 10^{-19} \times 2.69 \times 10^{16}}{60} = 2.439 \times 10^{-4}[\mathrm{J\ s^{-1}}] = 2.439 \times 10^{-4}\mathrm{W}.$$

Given the area of the cell surface equal to 2.26 cm^2, the energy of irradiation falling onto the area of 1 m^2 in 1 h (E_S) was calculated [W h m^{-2}].

$$E_S = 1.08\mathrm{W\ m^{-2}s} = 3888\mathrm{Whm^{-2}}.$$

As shown in Figure 1, the irradiation caused changes in the UV spectrum of the compound studied. The hipsochromic effect of the absorption band in the range 290–410 nm, appearing as a result of the irradiation, is assigned to the $\pi > \pi^*$ electronic transitions in the heterocyclic DHP ring. The hyperchromic effect in the range 250–290 nm, is related to the $\pi > \pi^*$ electronic transitions in the aromatic ring.

The photodegradation of manidipine was also analysed by the HPLC method and optimisation of the conditions of the analysis permitted a satisfactory separation of the products of decomposition. As follows from the results, two products of photodegradation were formed, characterised by the retention times $t_R = 21.39$ and $t_R = 23.16$ min – Figure 2.

An attempt at the qualitative identification of the photodegradation products was made with the use of a high-performance liquid chromatograph coupled with a mass detector. The ionisation was achieved by electrospray ionisation method. Because the method of electrospray ionisation leads to generation of protonated particles, the proper choice of the mobile phase is of key importance. A wrongly chosen mobile phase prevents correct recording of the spectrum. We have decided to use a mobile phase of methanol and water with addition of 10% formic acid.

The LC-MS mass spectra (Figure 3), reveal a series of peaks corresponding to the charged, protonated apparent molecular ions [M-zH$^+$]. In the range of the highest [M-zH$^+$] values, a peak corresponding to the protonated molecular ion was identified, which permitted a fast and accurate determination of the molecular mass of the compound.

The molecular ions corresponding to the compounds separated by HPLC were found at [M-zH$^+$] of 611 for MN, and at 609 and 595 for its photoproducts – nitrophenylpyridine and nitrozophenylpyridine derivative. Further analysis of mass fragmentation of the compounds formed indicated that the main photoproduct is the nitrophenylpyridine derivative formed as a result of aromatisation of the dihydropyridine ring. The concentration of the second product of photodegradation was about 20 times lower.

The analytical methods applied to assess the manidipine photodegradation permitted determination of the kinetic parameters of the process described by the relation $\ln c = f(t)$, including the reaction rate constant (k), half-life time ($t_{0,5}$) and time of decay of 10% of the compound ($t_{0,1}$).

The data presented in Table 2 and the results of earlier studies have shown that the photostability of manidipine is lower than that of the other DHP derivatives also having the NO_2 group at the meta position of the phenyl ring, such as nilvadipine or nimodipine. Quantitative assessment of manidipine photodegradation was also made on the basis of quantum yields of the photochemical reactions, which required measurements of the radiation energy absorbed by the system studied. These photochemical measurements were made by the commonly used method of chemical actinometry. The number of quanta emitted was made with the help of Reinecke salt, often used as a chemical actinometer. In the experiment the apparent quantum yields were measured and by extrapolation of the results to the zero time of irradiation (zero percent of substrate conversion) permitted determination of real quantum yield. For the majority of the known chemical reactions the quantum yield varies from 10^{-6} to 10^{-5}, and for primary processes φ is always 1. For manidipine the real quantum yields were of the order of 10^{-5}, which indicated the occurrence of secondary photochemical processes initiated by the primary products of decomposition. The quantum yield much lower than 1 is also characteristic of the reactions taking place in solutions, which is explained by deactivation of the excited molecules by the solvent (solvent quenching effect).

An attempt was also made at obtaining liquid inclusion complexes of manidipine with γ-CD. The stoichiometry of the complexes was determined by the Benesi-Hildebrand equation and the parameters obtained indicated the formation of 1:1 inclusion complexes characterised by the stability constant 3.39×10^4 (Figure 4). A comparison of the photochemical stability of manidipine in the inclusion complexes with that of its non-complexed form proved that in inclusion complexes its photochemical stability was only insignificantly higher.

Conclusions

Photochemical stability of manidipine in inclusion complexes with γ-CD is only insignificantly greater than that in its non-complexed form.

The main product of manidipine photodegradation is its nitrophenylpyridine derivative.

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