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Photodegradation of inclusion complexes of isradipine with methyl-β-cyclodextrin

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Abstract

The paper presents results of the studies on photochemical decomposition of isradipine (IS) and its liquid inclusion complexes with methyl-β-cyclodextrin (M-βCD). The process of photodegradation was assessed by the methods of UV spectrophotometry, HPLC (reverse-phase) and HPTLC (normal phase) chromatographic methods. The process of photodegradation of IS was analysed in the conditions of version I of the document of International Chemical Harmonization (ICH)-HBO-200 lamp. Quantitative evaluation of the photochemical decomposition was performed on the basis of the calculated photodegradation rate constant (k), half-life period ($t_{0.5}$) and time of degradation of 10% of the compound ($t_{0.1}$). Formation of inclusion complexes of IS with M-βCD was proved to increase twice the photostability of the drug. The analytical methods used were subjected to a validation procedure in which the limits of detectability and determinability as well as specificity, precision and sensitivity of the method were determined.
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Keywords: Isradipine; Methyl-β-cyclodextrin; Dihydropyridine calcium antagonists; High performance thin layer chromatography (HPTLC); Validation

1. Introduction

Isradipine (IS), like other 1,4-dihydropyridine derivatives (DHP) belongs to the recently synthesised group of calcium antagonists showing vasodilatory properties.

In particular it induces vasodilatation and weakens peripheral circulatory resistance [1–4].

IS is highly light-sensitive and its photodecomposition leads to molecular changes weakening the therapeutic effect [4–7].

This paper reports a fast and accurate UV method for simultaneous determination of IS and the products of its photodegradation. The spectrophotometric results were confirmed by HPLC and HPTLC measurements. As follows from earlier experiments, complexation of DHP derivatives with cyclodextrins (CD) increases the photostability of the former [8]. In the process of complexation apart from native cyclodextrins (α,

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β, γ), also alkyl and hydroxyalkyl derivatives of β-CD can be used.

In this study IS was complexed with methyl-β-cyclodextrin which is characterised by extremely well solubility in water [9–12].

2. Experimental

2.1. Materials

Methyl-β-cyclodextrin (M-βCD), mol. weight 1326.8 g/mol DS = 1.8 Wacker-Chemie GmbH; isradipine (IS), mol. weight 371.3 g/mol, Schwarz Pharma AG, 40789 Manheim. *n*-Hexan, 2-propanol, acetonitryl were of HPLC grade. All other chemicals and solvents were of analytical reagent grade.

2.2. Apparatus

All measurements were carried out on a Hewlett–Packard HPLC chromatograph, model 1050, equipped with a UV detector (working at 325 nm) and an integrator-II 3396 series. Spectrophotometric measurements were conducted on a Shimadzu UV-160 A spectrophotometer.

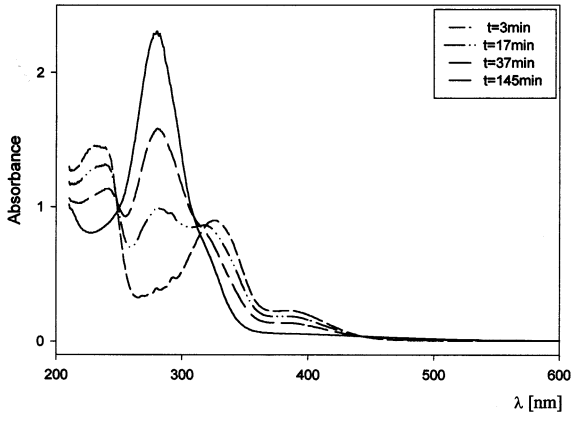

Fig. 1. UV spectra of the inclusion complex of isradipine with methyl-β-cyclodextrin after different time of photodegradation.

2.3. Inclusion complexes in solution

Inclusion complexes in solution of IS with M-βCD were determined using the method of Higuchi and Connors.

In this investigation, an excess of solid isradipine (50 mg) was added to a screw-capped tube (25 ml) containing ligand solution of the appropriate concentration from $1.5–13.5 \times 10^{-3}$ mol M-βCD/L. Then the samples were supplemented to the volume of 10.0 ml with Britton–Robinson buffer of pH 6.57 and shaken for 20 h at 37°C. For the samples to reach equilibrium they were kept in store for 4 days at 4°C, and then filtered off through a membrane Whatman filter with pores 0.65 μm in diameter. The concentration of IS in the filtrate was determined by the spectrophotometric method. The phase diagrams of solubility were drawn as dependencies of the dissolved IS on increasing concentration of M-βCD [13].

2.4. Photochemical stability in solution

2.4.1. Spectrophotometric study

A solution of the crystalline IS and its inclusion complexes, containing IS at a concentration 1.42×10^{-4} mol/l was used for the study. The solution was transferred to a cylindrical quartz cuvette ($V = 2.4$ ml), exposed to radiation for 200 min. The samples were irradiated in the conditions recommended in the version I of the International Chemical Harmonization (ICH). The irradiation was performed with an HBO-200 lamp and the maximum absorption was obtained using the Wood and interference filters. After the proper time of exposure, UV spectra were recorded in the range of 200–400 nm. Absorbency of the solutions was measured against methanol at the wavelength of $\lambda = 325$ nm ($l = 1$ cm).

Changes in the UV spectra of the inclusion complex of IS with M-βCD after different times of irradiation are illustrated in Fig. 1.

2.4.2. High performance liquid chromatography (HPLC)

IS and its photodegradation products were separated with a LiChrospher 100 RP-18 column

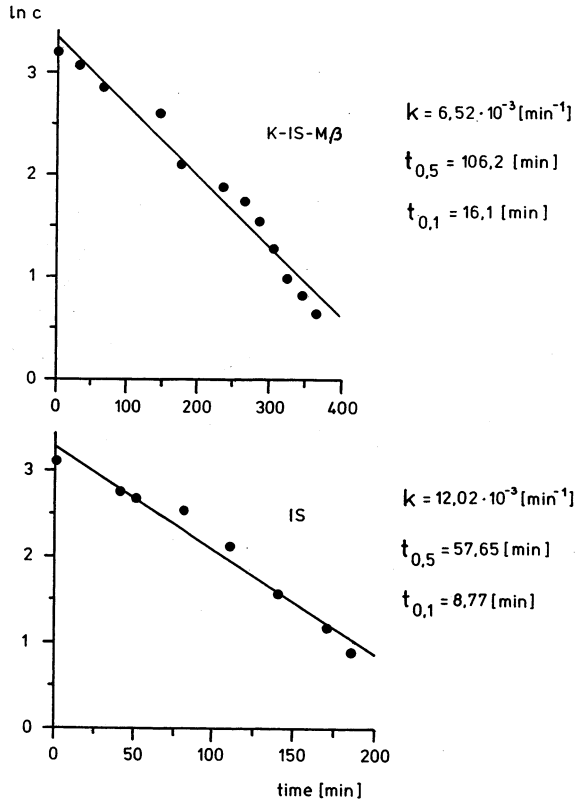

Fig. 2. The decomposition curves of isradipine (IS) and its inclusion complex with methyl-β-cyclodextrin (K-IS-Mβ): k = the rate constant of photodegradation; $t_{0.5}$ = the half-life degradation time; $t_{0.1}$ = the time of the decomposition of 10% compound.

(250 mm × 4 mm; 5 μm). The mobile phase consisted of methanol: water: acetonitrile at the ratio of 7:3:4.2 (v/v), and was flown at a rate of 0.8 ml/min. The injection volume was 20 μl; the column worked at room temperature.

Solutions of IS and its liquid inclusion complexes containing 6.1×10^{-5} mol/l were subjected to photochemical decomposition in the same conditions as described in Section 2.4.1.

The chromatograms of liquid inclusion complex of IS with M-βCD after irradiation are presented in Fig. 2.

2.4.3. High performance thin layer chromatography (HPTLC)

IS solutions after a certain time of photodegradation were placed on silica gel 60 F_{254} (Merck) plates size 10 cm × 10 cm. The chromatograms were developed in the classical chamber in the conditions of saturation with the mobile phase. The spots in the chromatograms were viewed in the light of UV lamp at $\lambda = 254$ nm. The mobile phase was a mixture of hexane: acetone: ethyl acetate /4: 2.5: 2.2 (v/v)/. The values of R_f and retention time of IS and the products of its photodegradation are shown in Table 1.

2.5. The kinetic parameters

Changes in the concentration of IS during irradiation were described by the equation:

$$\ln c = \ln c_o - k_{ob} \cdot t$$

where c is the concentration of the IS, k is the photodegradation rate constant, t is the time of the photodegradation. The results obtained are presented as the dependencies $\ln c = f(f)$.

On the basis of the above equation the following kinetic parameters were estimated:

- the rate constant of photodegradation (k)
- $t_{0.5}$ = the half-life time

Table 1
Chromatografic parameters (HPTLC and HPLC method) of inclusion complex of isradipine with methyl-β-cyclodextrin (IS) and its photodegradation products

Time of photodegradation (min)	The value of R_f		Retention time (min)	
	IS	Photodegradation products	IS	Photodegratation products
0	0.76	–	0.56	–
40	0.76	0.63, 0.50, 0.31, 0.27	0.56	3.5, 3.8, 4.4, 5.2
110	0.76	0.63, 0.50, –, 0.27	0.56	3.5, –, 4.4, 5.2

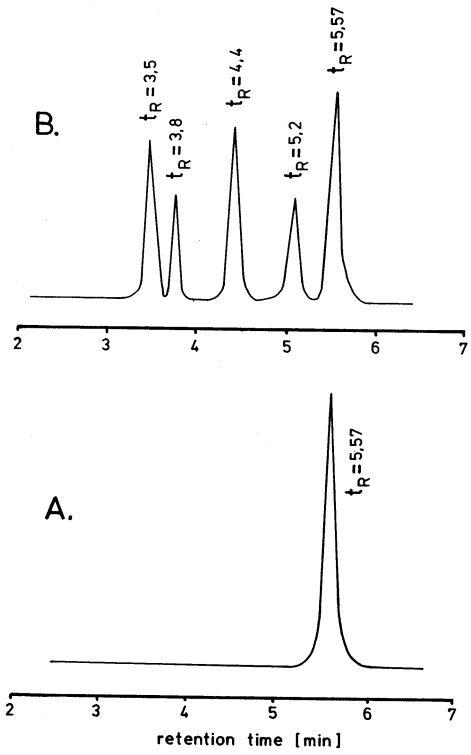

Fig. 3. The chromatograms of inclusion complex of isradipine with methyl-β-cyclodextrin after different time of photodegradation: A = 0 min; B = 40 min.

Table 2
Validation of the spectrophotometric and HPLC method for the determination of isradipine-precision

	Spectrophotometric method	HPLC
n	8	8
$\bar{x}$	6.78×10^{-5}	7.13×10^{-5}
S^2	1.14×10^{-14}	1.92×10^{-12}
S	1.07×10^{-7}	1.39×10^{-6}
S_x	3.78×10^{-8}	4.90×10^{-7}

- $t_{0.1}$ = the time of degradation of 10% of the drug

The first-order rate constants were calculated from the slope of the linear plots of $\ln c$ versus time of photodegradation.

The decomposition curve and the kinetic parameters of photodegradation of inclusion complex of IS with M-βCD are given in Fig. 3.

2.6. Validation

2.6.1. Linearity

The linear regresion analysis of IS was made by plotting the absorbance (spectrophotometric method) and the peak area (y) versus IS concentration (x) in mol/l. The calibration curve was constructed in the range 7.0×10^{-6}–14.0×10^{-5} mol IS/l. The following equations were obtained:

spectrophotometric method

$y = 1.4\times10^{4}x - 0.0182 \qquad r^2 = 0.9997$

HPLC

$y = 6.5\times10^{10} - 0.9\times10^{2} \qquad r^2 = 0.9994$

2.6.2. Detectability

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

spectrophotometric method

$\mathrm{LOD} = 0.35\times10^{-6}$ mol/l

$\mathrm{LOD} = 0.17\times10^{-6}$ mol/l

HPLC

$\mathrm{LOQ} = 3.50\times10^{-6}$ mol/l

$\mathrm{LOQ} = 0.52\times10^{-6}$ mol/l

2.6.3. Precision

The parameters characterising the precision of the HPLC and spectrophotometric method are given in Table 2.

3. Results and discussion

Drugs from the group of 1,4-dihydropyridine derivatives have been used for treatment of coronary heart disease and their activity is based on blocking of calcium ions access to the cardiac muscle [14]. Recently, more interest is paid to the second generation derivatives which are drugs of preferable activity on smooth muscle of blood vessels including isradipine. It is known that DHP derivatives are photolabile and products of their photochemical degradation have no therapeutic value [15,16]. Improvement of photostability of this group of drugs has been the subject of great interest. This study was undertaken to assess the process of photodegradation

of one of the latest DHP derivatives, IS, containing the oxadiasole group in the phenyl ring.

IS solution was subjected to UV irradiation and after a certain time intervals the UV spectra were taken. As shown in Fig. 1, as a result of the photodegradation process, a new absorption band appeared at 278 nm, moreover, two isosbestic points were observed at 312 and 248 nm.

The quantitative evaluation of the IS photodegradation process could not be performed on the basis of results of the spectrophotometric study as they gave no decisive information indicating whether it was a single-stage or two-stage process. Therefore, the photodegradation of IS was evaluated by HPLC using the reversed phase technique (RP-18) [17]. Optimisation of the chromatographic process proved that the best mobile phase was a mixture of methanol:water:acetonitrile (7:3:4.2 (v/v)). After certain time of photodegradation, the changes appearing in the chromatograms were analysed. With increasing time of photodegradation, the area of peaks attributed to the parent compound was gradually decreased. As shown in Fig. 2, after 40 min of irradiation, IS chromatograms revealed the presence of four products of photodegradation characterised by the retention times of 3.5; 3.8; 4.4 and 5.2 min. In the chromatogram taken after 110 min of irradiation the peak corresponding to the product of the retention time 3.8 min was observed to decrease while that corresponding to the product of the retention time 3.5 min increased which indicates the occurrence of the consequent reaction.

The peaks attributed to the degradation products of IS corresponded to compounds characterised by shorter retention times which means that these products are more polar than the parent crystalline drug. The results obtained by HPLC method were verified by HPTLC study [18]. There was a very good correlation between the results obtained by these two methods the number of peaks in the HPLC chromatogram attributed to the products of IS photodegradation was the same as the number of spots on the TLC chromatographic plate.

As mentioned above, DHP derivatives are an exceptionally photolabile group of drugs and it has been proved that their complexation with cyclodextrins improves their photostability. In this work M-βCD was chosen for complexation as it is significantly less polar and better water soluble than the native β-CD. The phase diagram obtained was of type A according to the classification accepted by Higuhi and Connors, which indicated the formation of the 1:1 inclusion complexes of IS with M-βCD. The rates of photodegradation of IS bound in complexes with M-βCD and in the crystalline form were compared.

Quantitative evaluation of the photochemical degradation was performed on the basis of the calculated values of rate constants of photodegradation (k), half-life time ($t_{0.5}$) and time of degradation of 10% of the compound ($t_{0.1}$). These parameters are important for the pharmaceutical point of view. The presence of M-βCD did not affect the character of IS photodegradation as there were no differences in the UV spectra and chromatograms of IS in the crystalline form and in inclusion complexes, however, a comparison of the rate constants of photodegradation, given in Fig. 3, revealed that IS complexation with M-βCD increased twice the photostability of the drug.

The analytical methods used were subjected to a validation procedure in which the limits of detectability and determinability as well as specificity, precision and sensitivity of the method were determined [19]. As follows from an analysis of the ranges of linearity, the correlation coefficients obtained in both cases were high. A comparison of the direction cosines has proved that the HPLC method is characterised by greater sensitivity, but unfortunately also a lower precision, than the UV spectrophotometry.

Acknowledgements

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