International Journal of Pharmaceutics, 80 (1992) 109–117

IJP 02654

Photostability evaluation of nicardipine · HCl solutions *

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(Received 9 August 1991)
(Accepted 12 September 1991)

Key words: Nicardipine · HCl; Photostability; Mass spectrometry; HPLC

Summary

A stability high-performance liquid chromatographic (HPLC) method is proposed as suitable to assess the photodegradation of nicardipine · HCl solutions. The method was validated with particular regard to selectivity. The application of such a procedure to the study of the degradation rate of nicardipine · HCl under both UV and daylight conditions is illustrated. Further structural studies were performed, by means of mass spectrometry, on the main degradation product collected from HPLC: this allowed its identification as the pyridine analogue of nicardipine.

Introduction

Nicardipine · HCl (2-(*N*-methylbenzylamino) ethylmethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine 3,5-dicarboxylate hydrochloride) is a calcium-channel blocking agent with coronary and peripheral arterial vasodilatatory activity. It is therefore effective in the treatment of angina and of mild to moderate hypertension. Its structure and activity are similar to those of nifedipine, but in the case of nicardipine the salt form is stable and is in use (Martindale, 1989).

Several kinds of preparations have been proposed for the administration of nicardipine · HCl. Tablets, capsules and coated pellets have been suggested for use as oral extended release formulations. Moreover, ointments and patches for transdermal administration and an intranasal spray solution have been studied (Takuzo et al., 1988). Among the nitrophenyl dihydropyridine derivatives, nicardipine has also been proposed, as an eye-drop formulation, for the treatment of glaucoma (Corbiere, 1987).

Although the problem of drug stability must always be taken into account, in the case of formulations either based on drug solution, or requiring a dissolution step during their preparation, particular care is of course necessary. For solid dosage forms, an awareness of the stability characteristics of a drug solution is essential for correctly performing control tests such as monitoring dissolution. This is of major concern in the

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* This work was presented in part at the V Convegno Nazionale di Analitica Farmaceutica, Farmachimici, 20–21 November 1990, Milano, Italy.

case of extended release preparations, for which dissolution tests are likely to last several hours.

Recently, several papers have been published concerning the study of the photodecomposition of nifedipine, a process that is well known to be characterized by extreme sensitivity to both UV and daylight (Testa et al., 1979; Pietta et al., 1981; Matsuda et al., 1989). The dihydropyridinic ring and the nitrophenyl function have been shown to react readily to yield the 4-(2′-nitrosophenyl)pyridine and 4-(2′-nitrophenyl)pyridine homologues, after exposure to UV and daylight. Such photolability makes it necessary for nifedipine to be handled under conditions involving considerable expense and the discomfort of controlled illumination.

Given its structural analogies with nifedipine, a certain sensitivity to light could also be envisaged for nicardipine · HCl, even though to our knowledge no specific information is as yet available in the literature on this subject. On the other hand, elucidation of the stability characteristics of nicardipine · HCl on exposure to light is essential in order to establish the precautions required during the development and control of dosage forms.

In order to acquire such knowledge, a simple isocratic HPLC method was developed and used to assess the photostability of nicardipine · HCl in both organic and aqueous media.

The method was validated with respect to its range of applicability and selectivity. The latter was assessed in the presence of the degradation products that were obtained according to a routine procedure, under conditions of forced degradation: peak homogeneity was verified by comparing the amount of drug determined at three different wavelengths and further confirmed via diode-array analysis (Wilson, 1990).

Materials and Methods

Nicardipine · HCl, as the β-crystalline form (Yan and Giunchedi, 1990), was purchased from Sifebat S.p.A., Tribiano (Milan, Italy).

HPLC analysis

Nicardipine-HCl samples were analyzed using an HPLC system (model 420 pump, I-459 integrator, model 432 UV-Vis detector, Kontron Instruments, Milan, Italy). The column was a Hibar Lichrosorb CN (10 μm) 250 × 4.0 mm, operating at room temperature and a flow rate of 1 ml/min. The mobile phase consisted of a mixture of 0.01 M sodium phosphate buffer at pH 6.1 and CH_3CN (1:1). 100-μl samples were injected; detection was performed at 240 nm.

The area of the peaks was used to calculate sample concentrations by reference to a calibration curve. No internal standard was employed.

The selectivity of the method was evaluated following exposure to both UV and daylight by comparing the amount of nicardipine determined at three different wavelengths (240, 260 and 350 nm) as described by Chow and Shah (1987); at each wavelength, a calibration curve was constructed to which partially degraded samples were referred.

Moreover, the sample exposed to UV light was analyzed by means of a diode-array system (HP 1090 M, 300 series Liquid Chromatography).

Photodegradation and isolation of degradation products

Nicardipine · HCl methanolic solutions of 1 mg/ml concentration were exposed to UV light (germicidal lamp: 250 nm, 40 W). Aqueous solutions of 0.1 mg/ml concentration were exposed to daylight. Exposure was performed in 10 mm quartz cuvettes containing about 3 ml of accurately weighed sample; solvent evaporation, which was very limited for the use of a plug on the cuvette, was nevertheless corrected on the grounds of weight just before each sampling. Samples were taken after 2, 4, 6, 8, 10 and 24 h of UV exposure, and after 7 and 14 h of daylight exposure; samples were diluted to a 10 μg/ml concentration with the mobile phase and, unless immediately injected, stored in the dark and at −20°C.

The isolation of the main degradation peak was carried out from the more concentrated methanolic solution extensively degraded by UV exposure: the same chromatographic method as

used for the analysis was employed for semi-preparative separations. The eluate corresponding to the main peak of the degradation product was collected, extracted with ethyl acetate, and dried under a nitrogen flow. Analysis of this sample was performed by mass spectrometry.

Mass spectrometry analysis

Electron ionization (EI) mass spectra and desorption chemical ionization (DCI) mass spectra were obtained with a Finnigan-Mat 8222 spectrometer via the direct inlet. Electron ionization was performed at 70 eV and 0.5 mA with a

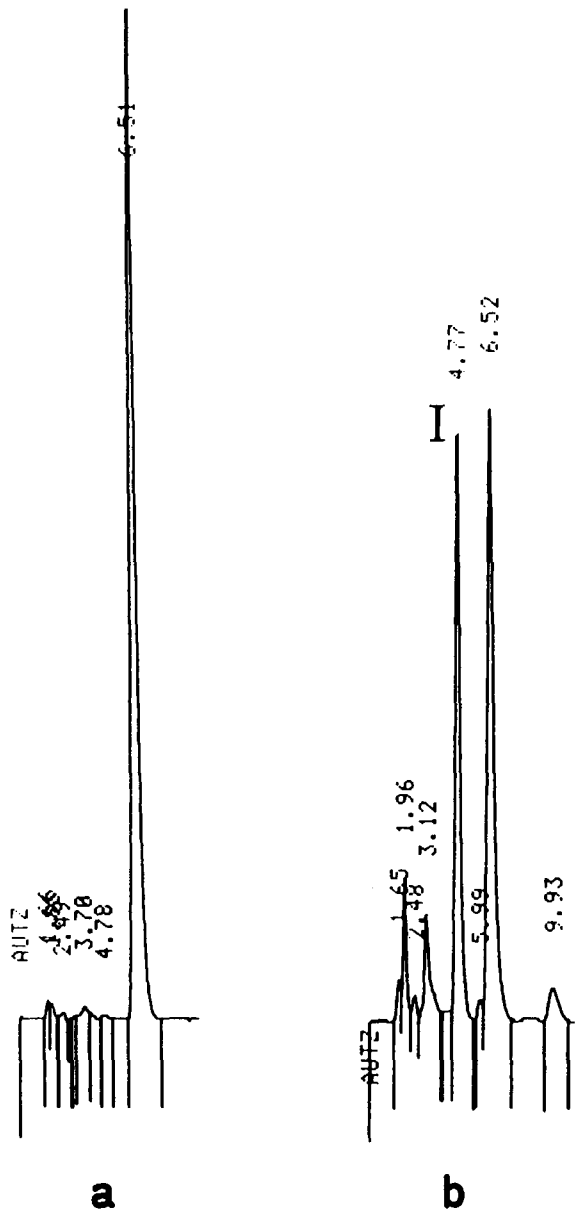


Fig. 1. Chromatograms of (a) nicardipine · HCl standard solution and (b) nicardipine · HCl sample degraded under UV light (different attenuations were used).

source temperature of 250°C. Accelerating voltage was 3 kV. The probe temperature was raised in two steps: from 20 to 180°C (0.2°C s^{-1}) and then from 180 to 350°C (1°C s^{-1}). Resolving power was 1200 (10% valley).

For desorption chemical ionization, ammonia (60 Pa, 160°C at the source) was used as ionization gas; the DCI emitter was heated by means of a 20 mA s^{-1} program up to 1 A.

Results and Discussion

Validation of HPLC method

In Fig. 1 the chromatograms of (a) a freshly prepared standard solution of nicardipine · HCl and (b) the same after 10 h UV irradiation are compared. The retention time of nicardipine was about 6.5 min. The samples degraded under different conditions of light exposure and in differing media (methanol and water) showed the same principal degradation peaks: in particular a major peak at a retention time of about 4.8 min (ascribed to product I) was present.

The linearity of the calibration curve was checked over the range between 0.5 and 20 μg/ml. The calibration curve and related parameters are illustrated in Fig. 2; the difference of the intercept from zero was found not to be significant ($P < 0.05$). The analysis of variance on the regression line is reported in Table 1: the F ratios confirm both the significance and the adequacy of the linear model.

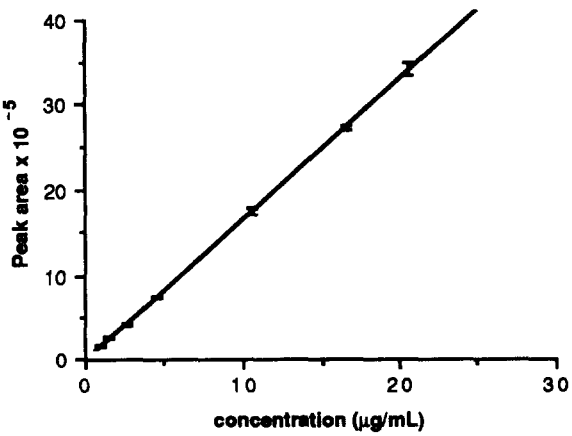


Fig. 2. Regression line and statistical parameters of calibration curve. Linear regression parameters: slope = 1.6500 (SE = 0.0090); intercept = −0.046 (SE = 0.0957); correlation coefficient = 0.9997 (23 degrees of freedom).

TABLE 1

Analysis of variance of the calibration line

Source	df	SS	MS	F_{ratio}
Regression	1	3605.49	3605.49	33872.4 [a]
Residual	23	2.448	0.106	
Lack of fit	5	0.124	0.0248	0.192 [b]
Pure error	18	2.324	0.129	

df, degrees of freedom; SS, sum of squares; MS, mean square.
[a] $33872.4 > F_{(1,23,0.95)}$: regression is significant.
[b] $0.192 < F_{(5,18,0.95)}$: lack of fit is not significant.

Tables 2 and 3, corresponding to the results for daylight and UV, respectively, show a statistical comparison between the concentrations of nicardipine calculated at 240, 260 and 350 nm: the lack of significant differences between the wavelength groups is in both cases indicative of peak homogeneity.

The results obtained by means of a diode-array detector are illustrated in Fig. 3. Both in the case of the unknown degradation product (Fig. 3a) and the nicardipine peak (Fig. 3b), the spectra at different times and the peak responses at four wavelengths (230, 240, 250 and 260 nm) were superimposable. This result further confirms the selectivity of the method and allows an assessment of its suitability for a degradation study of nicardipine · HCl based on determination of the amount of undegraded drug.

TABLE 2

Comparison between the amount of nicardipine · HCl after partial daylight degradation quantitated at 240, 260 and 350 nm (n = 3 for each wavelength)

Source	df	SS	MS	F_{ratio}
Between groups	2	0.017	0.008	0.295 [a]
Error	6	0.171	0.028	

df, degrees of freedom; SS, sum of squares; MS, mean square.
[a] $0.295 < F_{(2,6,0.95)}$: differences between groups are not significant.

TABLE 3

Comparison between the amount of nicardipine·HCl after partial UV degradation quantitated at 240, 260 and 350 nm (n = 3 for each wavelength)

Source	df	SS	MS	F_{ratio}
Between groups	2	0.004	0.002	0.564 [a]
Error	6	0.020	0.003	

df, degrees of freedom; SS, sum of squares; MS, mean square.
[a] $0.564 < F_{(2,6,0.95)}$; differences between groups are not significant.

Moreover, diode-array analysis also verified the peak homogeneity for the main degradation product I.

Mass spectrometry analysis

The EI mass spectrum of standard nicardipine is given in Fig. 4. Fig. 5 reports the mass spectra obtained with both EI and DCI (a and b, respectively) on the collected samples of unknown peak I: the DCI spectrum clearly shows the $(M + H)^+$

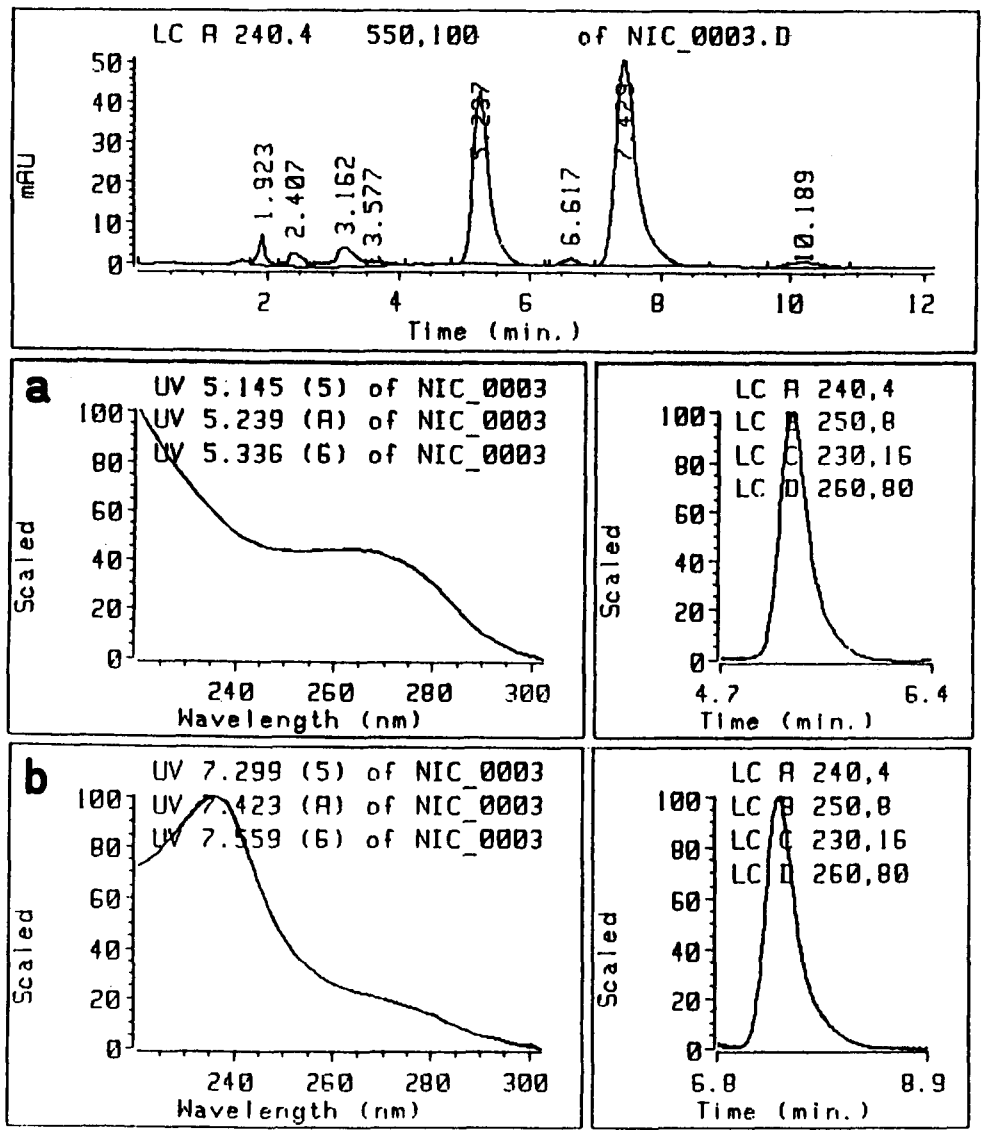


Fig. 3. Diode-array detection results relative to unknown degradation product I (a) and nicardipine peak (b).

peak at mass number m/z 478, from which a molecular weight of 477 was calculated. In Table 4 the major fragments of standard nicardipine and degradation product I are compared. The mass number 147 can be explained in both cases on the basis of a McLafferty rearrangement. A difference of 2 m/z units is observed in all the fragments whose structure includes the dihydropyridinic ring, while the same mass numbers characterize the fragments derived from the side chains. It is possible to argue that photodegradation also gives rise to the pyridinic derivative for nicardipine · HCl, in agreement with reported data for nifedipine and other related compounds (Squella et al., 1990).

Degradation kinetics

The results obtained on the degradation kinetics of methanolic solution are shown in Fig. 6. One can deduce from the plot shown that a first-order process is involved.

The results of a preliminary study in aqueous solution under daylight are listed in Table 5: slight but statistically significant degradation is evident.

Conclusions

The stability-indicating properties of the method described, arising from the good separation of unmodified nicardipine · HCl from photodegradation products, were proved by detection at different wavelengths and diode-array analysis. This result indicates that the method is suitable not only for degradation studies, but also in quality control on pharmaceutical dosage forms.

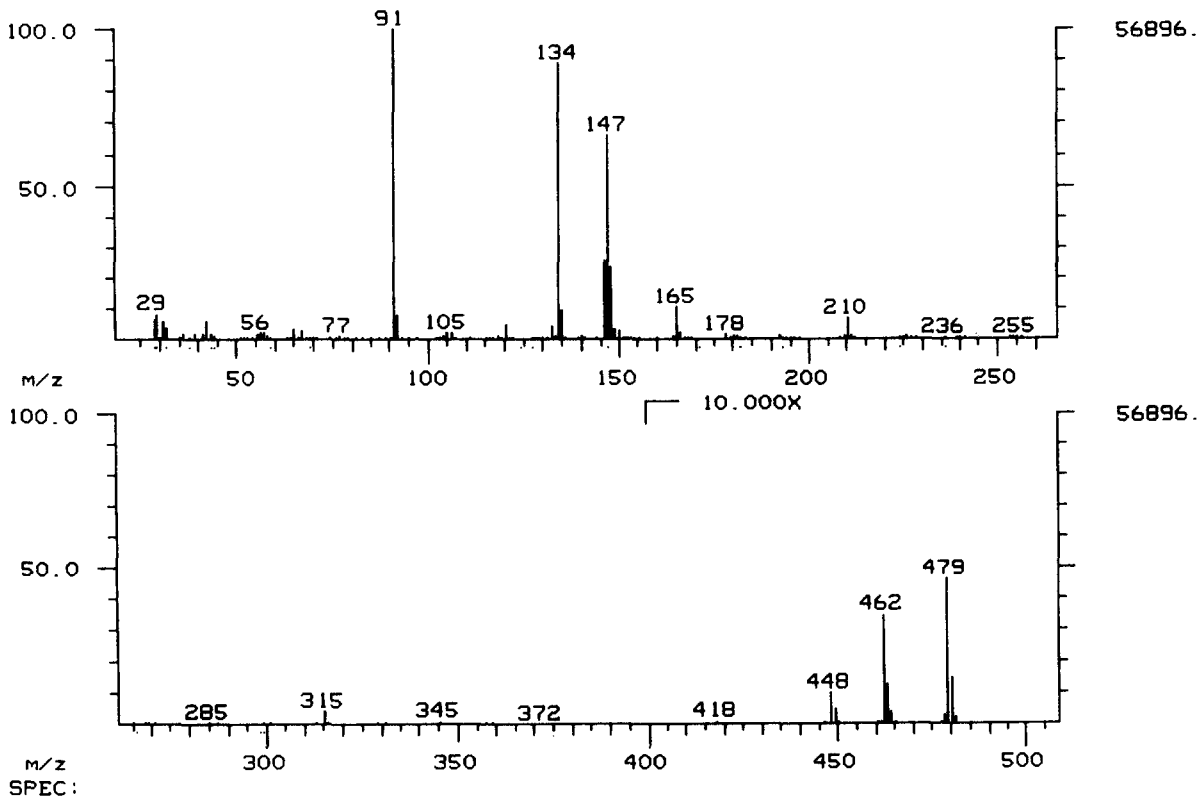


Fig. 4. Electron ionization mass spectrometry analysis of standard nicardipine · HCl.

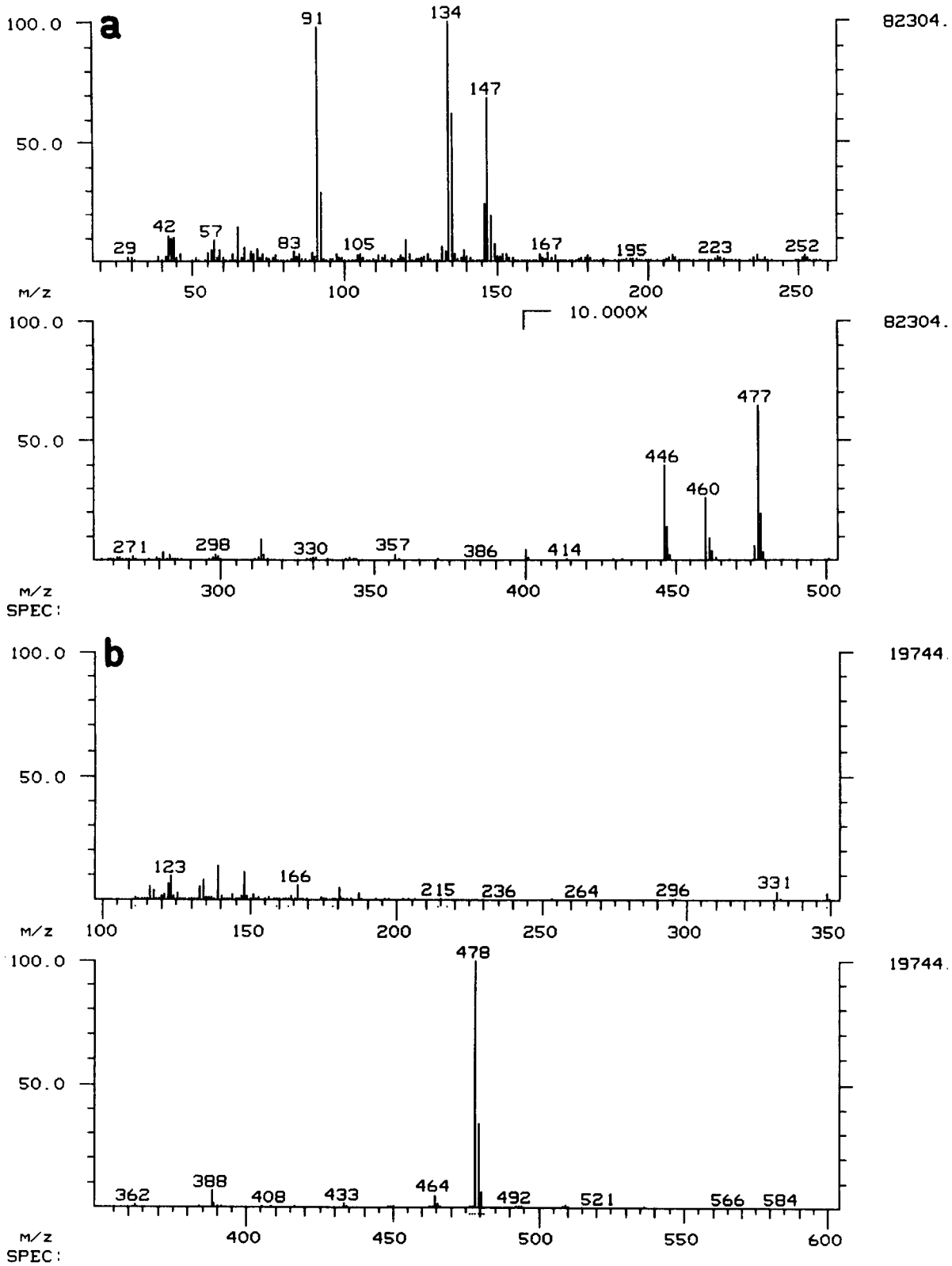
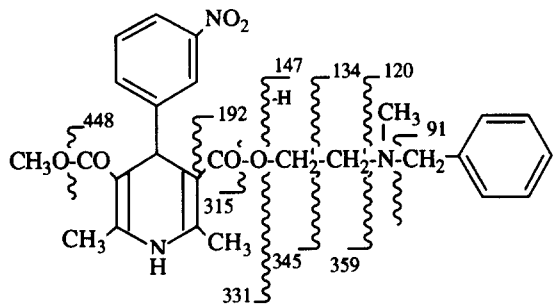


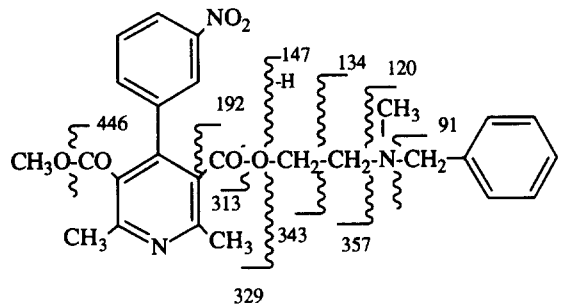
Fig. 5. Electron ionization (a) and desorption chemical ionization (b) mass spectrometry analysis of unknown degradation product I

TABLE 4

Comparison of significative mass spectrometry data of standard nicardipine (a) and degradation product I (b)



a



b

MS fragment	*m/z*	
	nicardipine	product I
M^+	479	477
M-OH	462	460
M-OCH_3	448	446
M-$N(CH_3)C_7H_7$	359	357
M-$CH_2N(CH_3)C_7H_7$	345	343
$C_2H_3N(CH_3)C_7H_7$	147	147
$CH_2N(CH_3)C_7H_7$	134	134
$N(CH_3)C_7H_7$	120	120

The degradation measured in aqueous solution suggests that nicardipine · HCl, although light sensitive, is less dramatically modified than nifedipine, and the usefulness of particular precautions must be critically assessed. The importance of photodegradation during, for example, a dissolution test, even in the case of prolonged release formulations, is likely to be quite low, especially considering the protection arising from the dissolution medium itself.

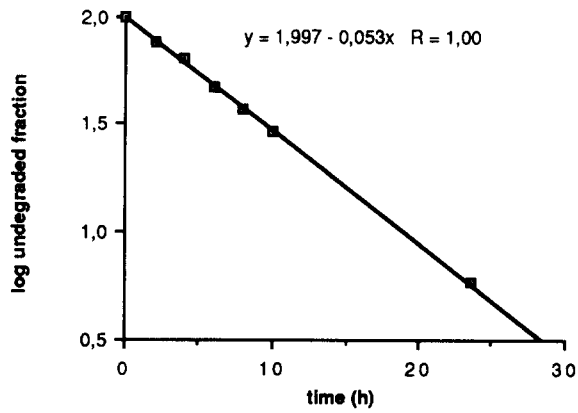


Fig. 6. Degradation kinetics of methanolic solution of nicardipine · HCl under UV light.

Nevertheless, an extent of 20% degradation after 14 h should be taken into account if a solution is to be used as a pharmaceutical dosage form, as in the case of eye drops or analogous preparations. In such cases, the use of protection against light should be considered.

Acknowledgements

The authors wish to thank Dr A. Zanotti Gerosa and Dr R. Prelini (Bayer Italia, Milano) and Dr A. Rava (IBP, Pavia) for helpful suggestions, and Dr M. Kitsos (Department of Pharmaceutical Chemistry, University of Pavia, Pavia) for the performance of diode-array analysis. This work was partially supported by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST).

TABLE 5

Undegraded nicardipine · HCl in aqueous solution after daylight exposure (means ± SD of three replicates)

Time (h)	Concentration (μg/ml)	Percent undegraded
0	9.84 ± 0.040	–
7	9.07 ± 0.032 [a]	92.2
14	7.80 ± 0.025 [a]	79.2

[a] Values significantly different from the initial concentration according to a *t*-test ($P < 0.01$).

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