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Determination of degradation products from the calcium-channel blocker isradipine

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Abstract

Mass Spectrometry has been used to determine the identity of a number of degradation products from the bulk drug form of Isradipine (DynaCirc). Liquid chromatography coupled with mass spectrometry (LC/MS) was used to analyze the degraded samples and tentative identifications were made based upon the known reactivity of the molecule, molecular weight measurements and mass spectral fragmentation patterns. Isradipine was found to be stable to heating, acidic and basic conditions, but susceptible to degradation from exposure to UV light and oxidative processes. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Isradipine; LC/MS; Pharmaceuticals; Degradation

1. Introduction

Isradipine [isopropylmethyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate] is a potent calcium channel antagonist of the dihydropyridine class. There are already several assays available for the analysis of this important group of drugs including gas chromatography/mass spectrometry (GC/MS) [1–4], high performance liquid chromatography (HPLC) [5–12], capillary electrophoresis [13], high performance thin layer chromatography (HPTLC) [14,15], electrochemical [16], and radioimmunoassay (RIA) [17]. There has also been a study conducted on the photodegradation products of nifedipine in hospital preparations by HPLC [18], as well as a study on the chromatographic purity of nicardipine by HPLC which describes a photodegradation product [19]. For isradipine, there are literature HPLC methods reported for both chiral [6,7,9,10,12], as well as racemic [5,8] separations from biological fluids and recently, there has even been a report of an

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analysis from the dosage form [11]. However, there have been no attempts to characterize the degradation products of isradipine. The present work describes the use of LC/MS to determine the structure for the degradation products of isradipine upon exposure to extremes in light, heat, pH and oxidizing agents.

2. Experimental

2.1. Samples

Isradipine was supplied by Swiss Pharma (Cairo, Egypt) as an authentic reference material.

2.2. Reagents and chemicals

Methanol (HPLC grade), acetonitrile (HPLC grade), sodium hydroxide, 3% hydrogen peroxide and concentrated hydrochloric acid were obtained from J.T. Baker (Phillipsburg, NJ) and were used without further purification.

2.3. Chromatography

Separations were carried out using a Hewlett Packard (Palo Alto, CA) Model 1100 HPLC system consisting of a vacuum degassing module Rheodyne Model 7725 manual injector equipped with a 20 μl loop, a quaternary pump, column heater and variable wavelength detector. Data acquisition was performed using the Hewlett Packard LC 2D ChemStation software operating on a PC. The column was an octadecylsilane stationary phase (Ultrasphere, 15 cm $\times$ 4.6 mm I.D., 5 μm particle size, Beckman Instruments, San Ramon, CA). The separation was affected using a gradient of acetonitrile–water starting at 25% acetonitrile and increasing to 40% over 22 min. The column temperature was maintained at 25°C and the flow rate was 1.0 ml min^{-1}. The detector wavelength was set at 254 nm.

2.4. Mass spectrometry

LC/MS experiments were carried out using a Micromass, (Beverly, MA) Quattro II triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. The needle voltage was approximately 3500 V and the cone voltage was held at approximately 60 V. Scans were acquired over the mass range from m/z 100–500 at 3 s per scan. MS data acquisition and analysis were performed using MassLynx NT version 2.22.

2.5. Degradation conditions

A stock standard of isradipine was prepared at a concentration of 1 mg ml^{-1} in methanol. For base degradation studies, 1 ml of the isradipine standard was added to 4 ml of 0.1 N NaOH in a scintillation vial which was sealed to prevent evaporation of solvent during heating. This solution was left at ambient temperature or heated at 60°C for 6 h. The solution was neutralized with a corresponding volume of 0.1 N HCl prior to analysis.

For acid degradation studies, 4 ml of 0.1 N HCl were added to 1 ml of the 1 mg ml^{-1} isradipine stock standard in a scintillation vial which was sealed. This solution was left at ambient temperature or heated at 60°C for 6 h. The acidified solutions were neutralized with a corresponding volume of 0.1 N NaOH prior to analysis.

For oxidative degradation studies, 4 ml of a 3% hydrogen peroxide solution were added to 1 ml of the isradipine stock standard in a scintillation vial which was sealed. This solution was left at ambient temperature or heated at 60°C for 6 h.

For UV degradation studies, 1 ml of the isradipine stock standard was diluted with 4 ml of methanol and placed in a scintillation vial, which was then placed inside a cabinet under a hand-held 254 nm UV lamp for up to 24 h.

3. Results and discussion

The electrospray mass spectrum of isradipine is shown in Fig. 1. One of the most interesting features of this spectrum of isradipine is the base peak, which is observed predominately as the sodiated molecular species $[M + Na]^+$ (m/z 394)

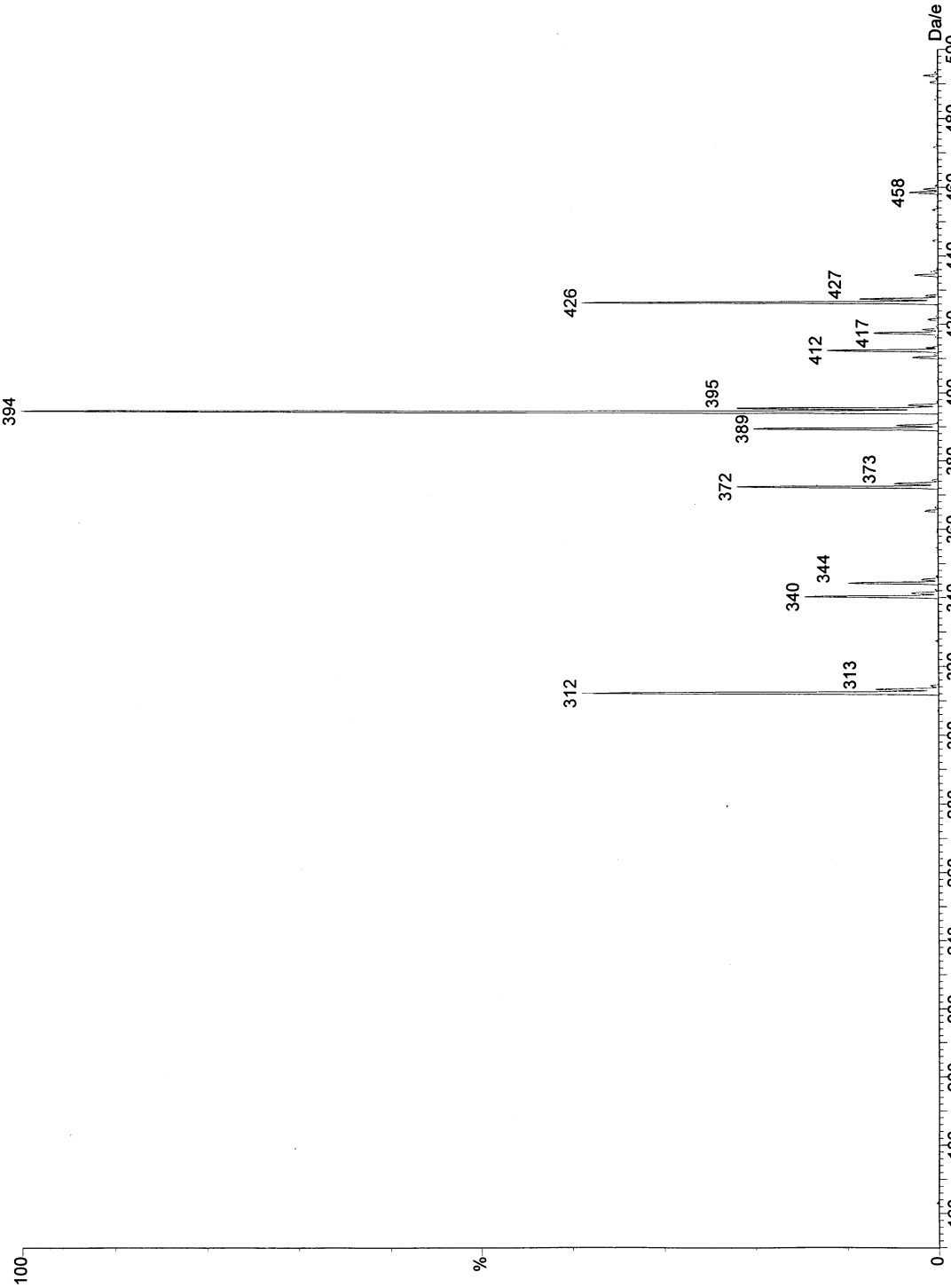


Fig. 1. Positive ion electrospray MS of isradipine infused at 10 μl min^{-1} at a concentration of 20 pmol ml^{-1} in methanol. Ion identities are as follows: m/z 372 $[M+H]^+$, m/z 389 $[M+NH_4]^+$, m/z 410 $[M+K]^+$, m/z 426 $[M+Na+CH_3OH]^+$ and two fragment ions m/z 340 $[M+H-CH_3OH]^+$ and m/z 312 $[M+H-IPA]^+$.

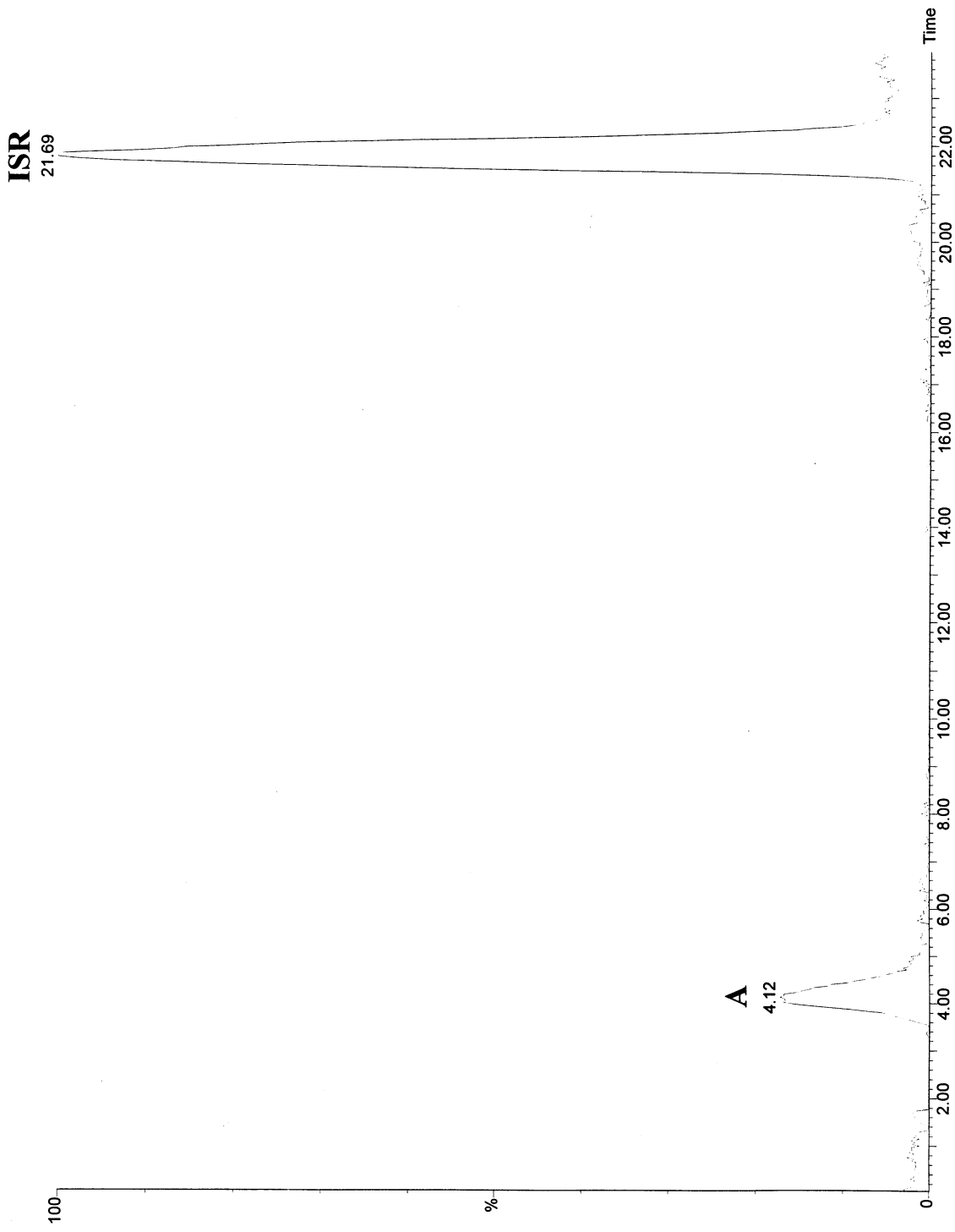
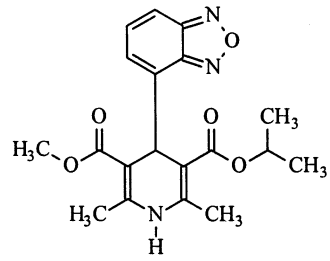
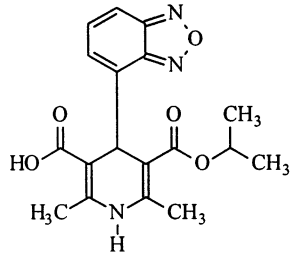


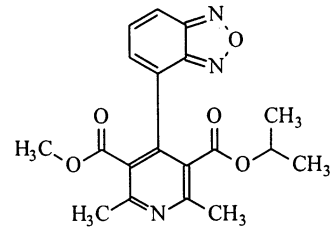
Fig. 2. LC/MS total ion chromatogram from the incubation of isradipine with sodium hydroxide for 6 h at 60°C.



ISRADIPINE



BASE DEGRADATION PRODUCT



UV DEGRADATION PRODUCT

Fig. 3. Structure of isradipine along with proposed base and UV degradation products.

versus the more normally observed $[M + H]^+$ (m/z 372). The sodiated species is observed in flow injection ESI experiments from 100% methanol, as well as LC/MS of the standard and all degradation studies. Surprisingly, most of the degradation products are represented primarily by the protonated molecular species, although all of the degradation products show some degree of sodium attachment. Therefore, it appears that isradipine possesses some particular affinity for sodium rather than this peak arising through a simple adduction process. There are also molecular species associated with the attachment of ammonia, potassium and methanol. These peaks (m/z 389 $[M + NH_4]^+$, m/z 410 $[M + K]^+$, and m/z 426 $[M + Na + CH_3OH]^+$ are generally observed with all of the degradation products and were invaluable in helping to establish the identity of the molecular species (i.e. if a certain peak corresponds to the $[M + H]^+$, then there should be a peak 17 mass units higher $[M + NH_4]^+$, 22 mass units higher $[M + Na]^+$, 38 mass units higher $[M + K]^+$ and 54 mass units higher $[M + Na + CH_3OH]^+$. In addition to the multitude of molecular species observed from isradipine, there are also two very diagnostic fragment ions. The first is the loss of 32 mass units observed at m/z 340 and the second is the loss of 60 mass units observed at m/z 312. These two losses are associated with the loss of methanol and isopropanol and represent the presence of the methyl and isopropyl esters. The presence of these two fragment ions are important indicators of state of these two functional groups in the degradation products. Other peaks present in the spectra are the $[M + Na + NH_4]^+$ at m/z 412, $[M + 2Na]^+$ at m/z 417, and $[M + Na + 2CH_3OH]^+$ at m/z 458.

Fig. 2 shows the LC/MS total ion chromatogram from the base degradation after 6 h of heating the reaction mixture at 60°C. Only one reaction product is detected at a molecular weight of 357. The loss of 14 mass units from isradipine is presumed to arise from the conversion of the methyl ester to the corresponding carboxylic acid (shown in Fig. 3). This assignment is further supported by the lack of the characteristic loss of methanol which would be observed if the methyl ester remained. Under ambient conditions, peak

A is still the only degradation product observed after 6 h and represents about 3% of the original total. Heating the solution causes a rise in the abundance to 15% (as seen in Fig. 2), but no additional peaks are observed. It is interesting to note that there is no evidence for the loss of the isopropyl ester moiety under basic conditions.

Isradipine appears to be stable to acid degradation. After 6 h of reaction time at ambient temperature, no degradation products were observed. Even after heating the reaction mixture at 60°C for 6 h, only 4% of the original sample degraded into a single component. Fig. 4 shows the LC/MS total ion chromatogram from the acid degradation after 6 h of heating the reaction mixture. The degradation product detected at a retention time of approximately 11 min has the same molecular weight as isadipine (371), but does not show the abundant fragment ions resulting from the loss of the two ester groups. The identity of this species is still unknown.

Fig. 5 shows the LC/MS total ion chromatogram from UV degradation after 24 h. A single major component is observed at a retention time of 20 min and comprises 20% of the original sample. This degradation product is formed through the conversion of the dihydropyridine ring to a pyridine ring via loss of H_2 (See Fig. 3 for structure). This product has been reported as the major photodegradation product from all of the dihydropyridine based calcium-channel blockers studied to date [18,19].

Fig. 6 shows the LC/MS total ion chromatogram from the peroxide degradation after 6 h of heating the reaction mixture at 60°C. In contrast to the other reactions, the peroxide degradation shows the formation of seven different reaction products. In this case, only 20% of the original sample remains after 6 h. However, even at ambient temperature, 50% of the sample was converted by this time. The proposed structures for the degradation products are shown in Fig. 7. Peak A (molecular weight 437) involves the opening of the benzoxadiazole ring in addition to oxidation of the nitrogen atom in the pyridine ring. Upon opening of the benzoxadiazole ring, it is proposed that the oxadiazole nitrogens are converted to a hydroxylamine and a nitro group. We also propose that oxidation of the pyridine ring occurs by formation of the N-oxide. Peak B has a molecular weight of 387 and is proposed to be a simple hydroxylation of the benzoxadiazole ring. The increase in molecular weight of 16 would tend to support this assignment. The presence of the characteristic fragment ions from the loss of methanol and isopropanol precludes the placement of the hydroxyl moiety on these functional groups. Therefore, it is proposed that this degradation product represents a hydroxylation on one of the three available positions of the phenyl ring. The major components, labeled as peaks C and D, appear to be isomers since they have the same molecular weight (421) and fragmentation patterns showing loss of methanol and isopropanol. We propose that these two isomers arise through oxidative opening of the benzoxadiazole ring forming the structures shown in Fig. 7. Peaks E and F are also isomers (molecular weight 406) and appear to arise through this same type of ring opening reaction, with the difference being a phenolic hydroxyl group replacing the hydroxylamine on one of the oxdiazole derived nitrogens. Peaks E and F also yield limited fragmentation, showing only the diagnostic losses of methanol and isopropanol. The final product, labeled as Peak G (molecular weight 369), arises through the conversion of the dihydropyridine ring to the aromatic pyridine form. This reaction product was also observed in the UV degradation studies.

It is interesting to note that in studies using MS/MS, mass spectra of isradipine and its degradation products show the predominate loss of the ester moieties, with little additional fragmentation observed, limiting its utility in the determination of the identities of the degradation products.

4. Conclusions

The calcium-channel blocker isradipine was found to be highly stable to acid degradation. Under basic conditions, conversion of the methyl ester to the corresponding carboxylic acid was found to occur under both ambient and 60°C temperatures. However, more than 85% of the original sample remained intact even under the

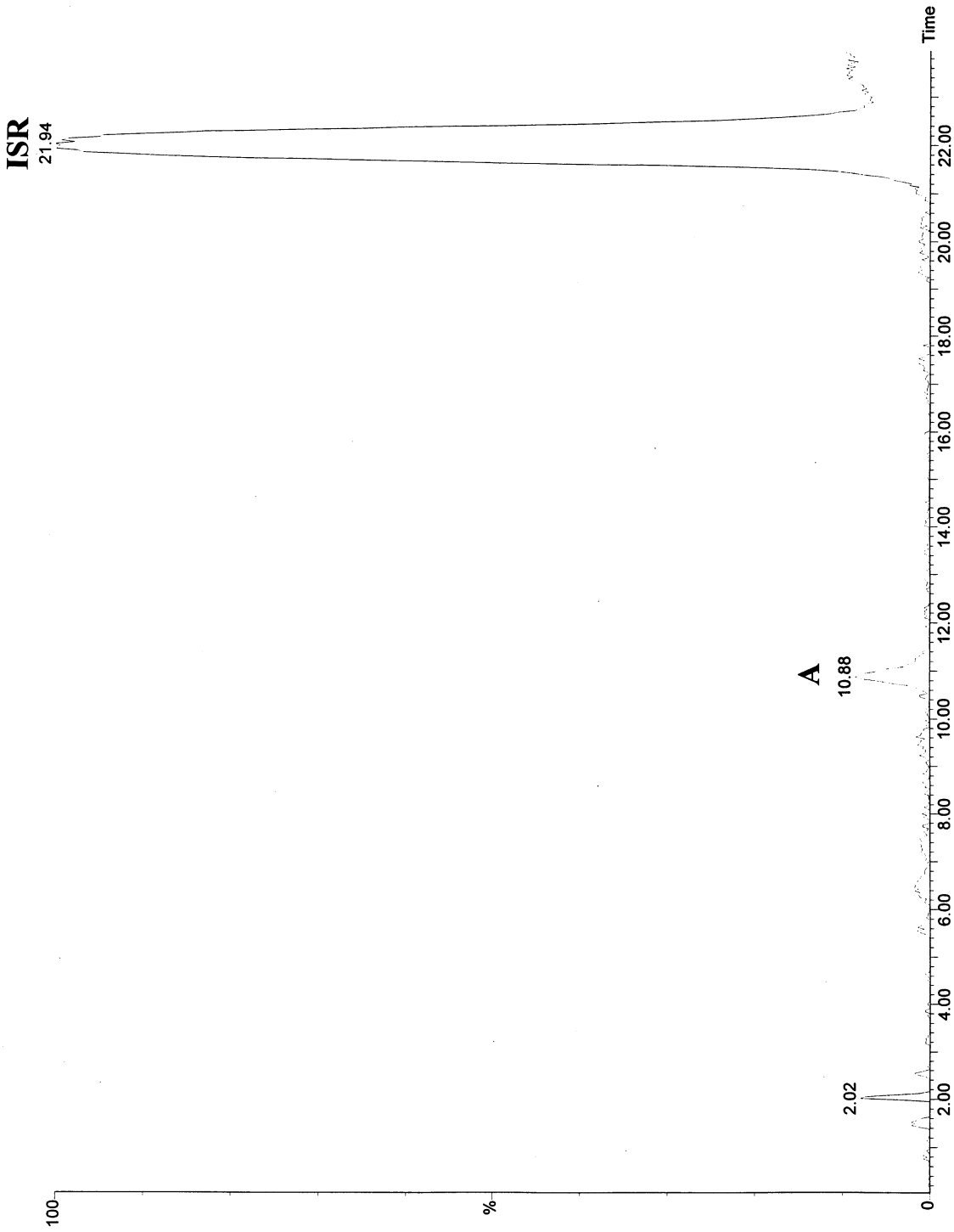


Fig. 4. LC/MS total ion chromatogram from the incubation of isradipine with hydrochloric acid for 6 h at 60°C.

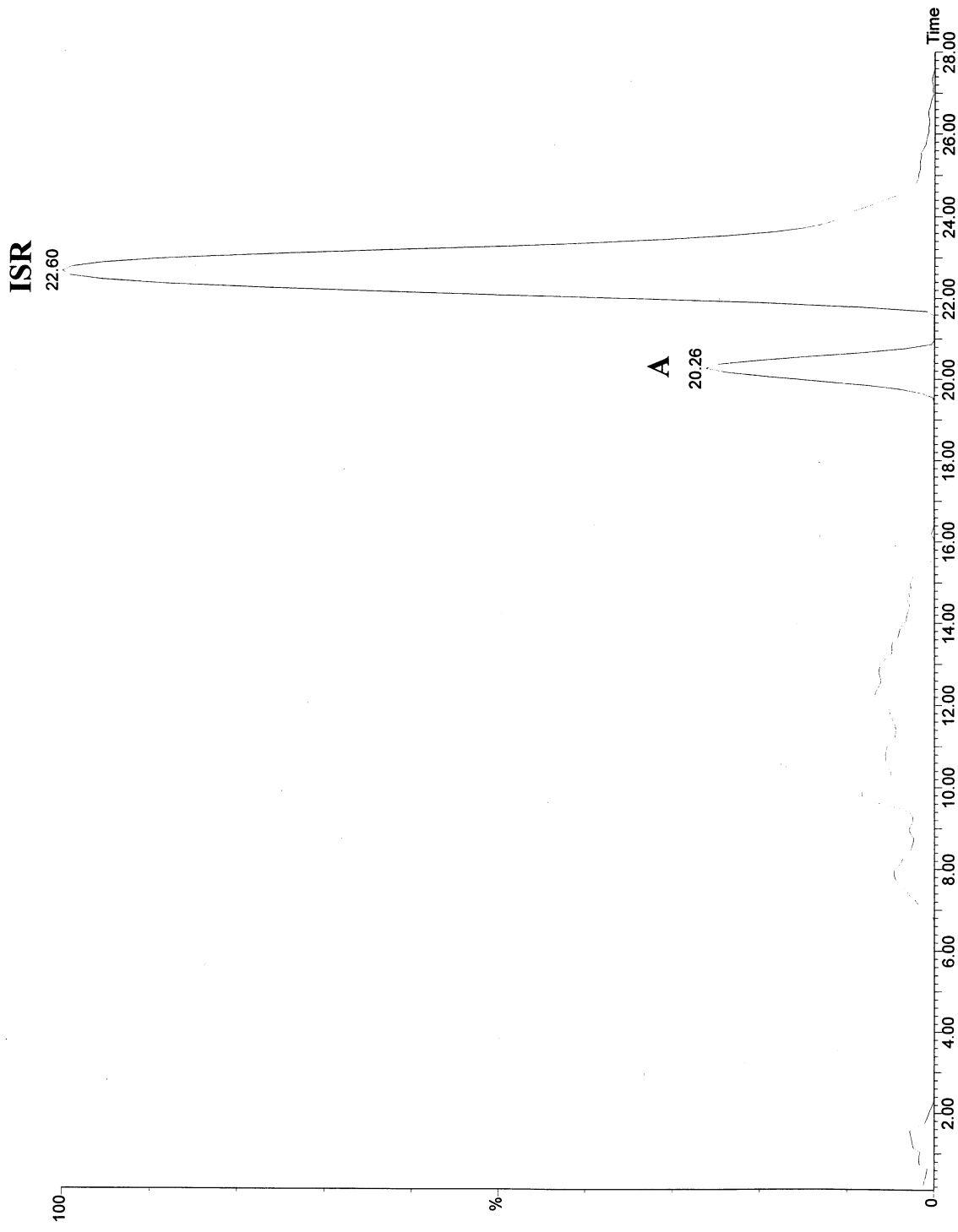


Fig. 5. LC/MS total ion chromatogram from the exposure of isradipine to UV radiation for 24 h at ambient temperature.

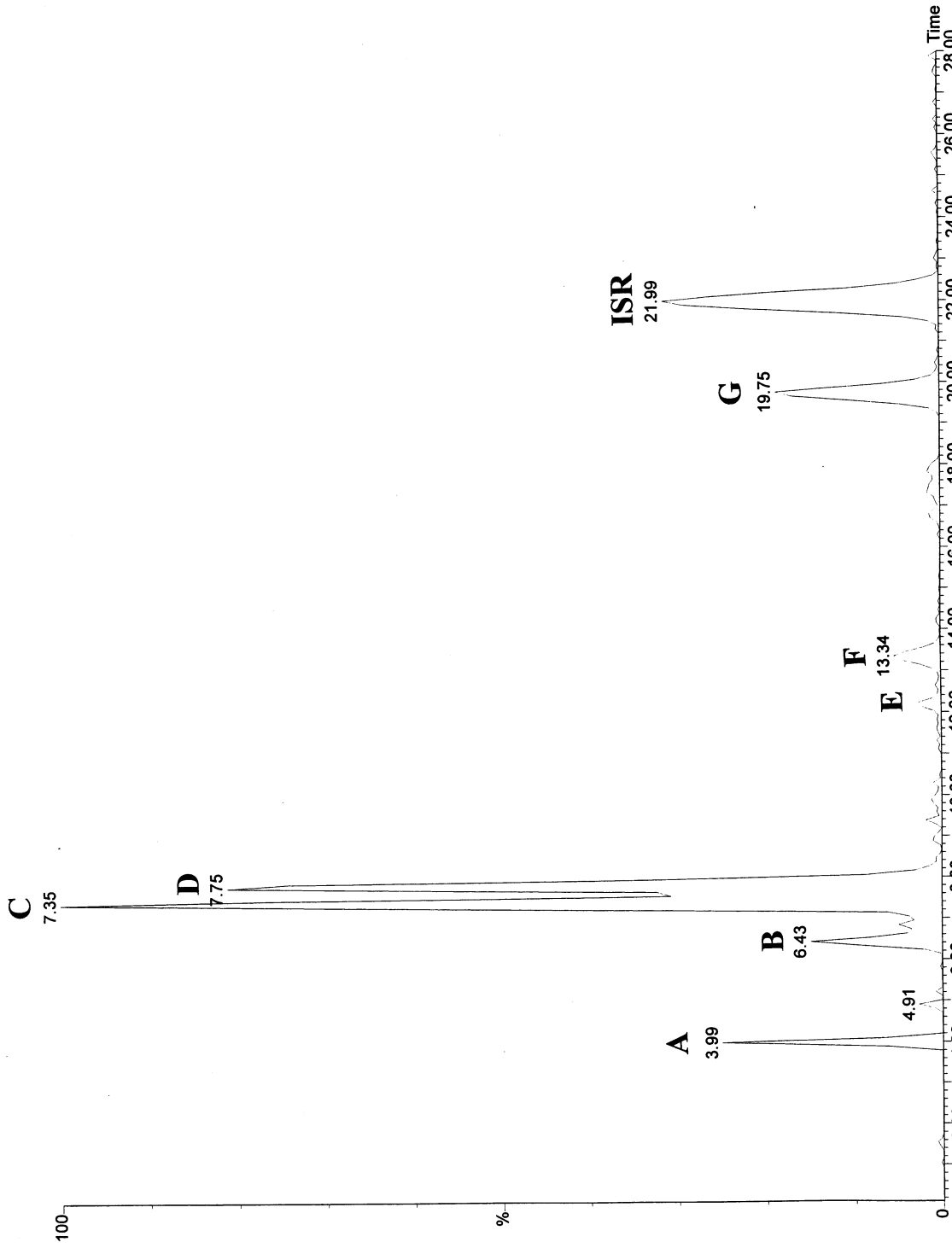
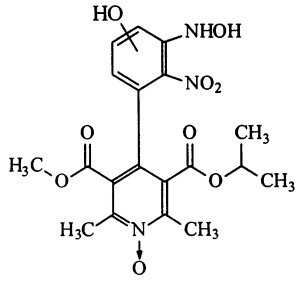
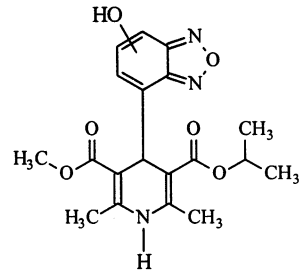


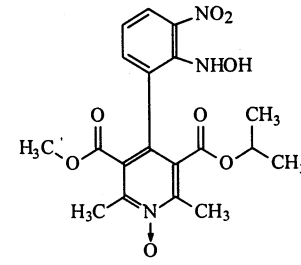
Fig. 6. LC/MS total ion chromatogram from the incubation of isradipine with hydrogen peroxide for 6 h at 60°C.



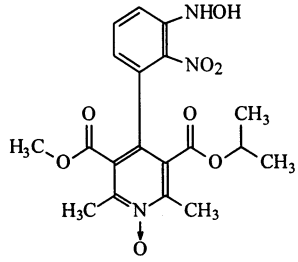
PEROXIDE DEGRADATION PRODUCT
PEAK A



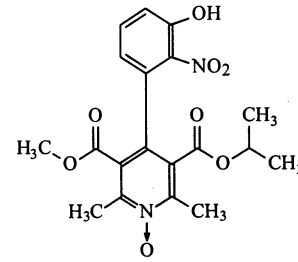
PEROXIDE DEGRADATION PRODUCT
PEAK B



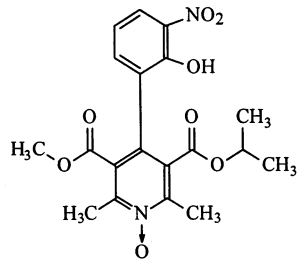
PEROXIDE DEGRADATION PRODUCT
PEAK C



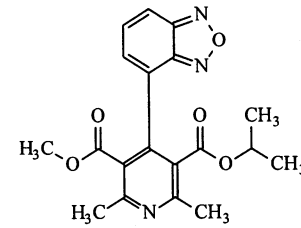
PEROXIDE DEGRADATION PRODUCT
PEAK D



PEROXIDE DEGRADATION PRODUCT
PEAK E



PEROXIDE DEGRADATION PRODUCT
PEAK F



PEROXIDE DEGRADATION PRODUCT
PEAK G

Fig. 7. Proposed structures for peroxide-induced degradation products from isradipine.

most extreme conditions tested. Exposure to UV radiation caused 20% of the sample to be converted from the dihydropyridine form to the pyridine analog within 24 h. Exposure to oxidizing conditions caused extensive degradation of the sample. After 6 h at ambient temperature, 50% of the original sample was converted to one of seven different degradation products. Repeating this

study at 60°C caused an additional 30% degradation but did not cause the formation of any additional degradation products. The package insert for isradipine states that the compound should be stored in a tightly sealed container and protected from light. Our findings support the need to follow these instructions when using this product.

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