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Jpn. J. Forensic Toxicol. Vol.11, No.1, 1993

原　　報

POSITIVE AND NEGATIVE ION MASS SPECTROMETRY AND RAPID EXTRACTION WITH SEP-PAK C_{18} CARTRIDGES FOR DIHYDROPYRIDINE CALCIUM ANTAGONISTS

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Received March 20, 1993
Accepted March 24, 1993

ジヒドロピリジン系カルシウム拮抗剤の正・負イオン質量分析とセップパックC_{18}カートリッジによる迅速分離法

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Summary

Positive-ion electron impact (PIEI), positive-ion chemical ionization (PICI) and negative-ion chemical ionization (NICI) mass spectra of nifedipine, nisoldipine, nitrendipine, dehydronitrosonifedipine and dehydronitrosonisoldipine, were presented, and each fragment mode was analyzed. In the PIEI mode, molecular cations appeared in all compounds, though they were small. In the PICI mode, $[M+1]^+$ quasi-molecular cations together with $[M+C_2H_5]^+$ ions and many fragment ions, appeared. In the NICI mode, the number of peaks was much less than that in both positive modes; intense molecular anions were observed for nifedipine, nisoldipine and nitrendipine, which seem very useful for sensitive and specific detection by gas chromatography (GC)/mass spectrometry (MS) in the NICI mode. Nifedipine, nisoldipine and nitrendipine, which had been added to body fluid samples, could be rapidly extracted by the use of Sep-Pak C_{18} cartridges. They could be detected both with and without photodecomposition by capillary GC with a DB-1 column, with satisfactory separation and recovery. We recommend the analytical method with photodecomposition, because no special care for light is required during the whole procedure.

Key words: Nifedipine; Nisoldipine; Nitrendipine; Dehydronitrosonifedipine; Dehydronitrosonisoldipine; Photodecomposition; Mass spectrometry; Gas chromatography; Sep-Pak C_{18} cartridges; Negative ion

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Introduction

Nifedipine, nisoldipine and nitrendipine are calcium antagonistic drugs commonly used in the treatment of cardiovascular diseases [1−3], and occasionally encountered in forensic science practice [4]. Nifedipine and nisoldipine are very sensitive to photodecomposition to be easily converted to dehydronitrosonifedipine and dehydronitrosonisoldipine, respectively [5, 6]. The present paper deals with positive-ion electron impact (PIEI), positive-ion chemical ionization (PICI) and negative-ion chemical ionization (NICI) mass spectra of nifedipine, nisoldipine, nitrendipine, dehydronitrosonifedipine and dehydronitrosonisoldipine. Prior to their actual identification by gas chromatography/mass spectrometry (GC/MS), a method for their rapid clean-up with Sep-Pak C_{18} cartridges is also presented.

Experimental

Chemicals

Nifedipine and nisoldipine were obtained from Bayer AG (Leverkusen, Germany); and nitrendipine from Yoshitomi Pharmaceutical Ind.Co., Ltd. (Osaka). Other common chemicals used were of the analytical grade. Sep-Pak C_{18} cartridges were purchased from Waters Associates (Milford, MA, USA); a DB-1 fused silica capillary column (15 m x 0.32 mm i.d., film thickness 0.25 μm) obtained from J & W Scientific (Folsom, CA, USA). Whole blood, serum and urine were obtained from healthy subjects.

MS conditions

Mass spectra in every mode were recorded by GC/MS on a JMS-AX505H MS instrument (JEOL, Tokyo) equipped with an HP-5890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA, USA) and the DB-1 capillary column. MS conditions were: accelerating voltage 3.0 kV, ionization current 300 μA, separator temperature 250 ℃; in the PIEI mode, electron energy 70 eV; in the PICI and NICI modes, electron energy 200 eV, reagent gas methane and chamber pressure 1 Torr. GC conditions for the GC/MS are the same as those described below for GC only.

GC conditions

GC analyses were carried out on an HP-5890 Series II gas chromatograph with flame ionization detection. The conditions were: injection temperature, 220 ℃; and helium gas flow, 3 ml/min. The samples were injected in the splitless mode and the splitter was opened after 1 min.

Extraction procedure

Sep-Pak C_{18} cartridges were pretreated by passing 10 ml of chloroform/methanol (9 : 1), 10 ml of methanol and 20 ml of distilled water. For new cartridges, this procedure was repeated more than twice to reduce background noise.

One milliliter serum or urine containing nifedipine, nisoldipine and nitrendipine (5 μg each) were mixed with 4 ml distilled water; in the case of whole blood, the 1 ml sample was mixed with 9 ml distilled water for complete hemolysis. The sample solution was poured into the pretreated cartridge. This was then washed with 20 ml distilled water followed by 3 ml chloroform/methanol (9 : 1) to elute the drugs from the cartridge. After discarding the aqueous layer (upper phase) with a Pasteur pipette, the organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl methanol and a 1—μl aliquot of it was subjected to GC analysis.

For measurements of unchanged forms of nifedipine, nisoldipine and nitrendipine, all above preparative procedure was carried out in the dark to prevent them from photodecomposition.

For measurements of the drugs in the forms of photodecomposition products, the above final residue dissolved in 100 μl methanol was exposed to a usual fluorescent lamp (400—600 nm, 4000 lux) for 60 min to complete photodecomposition; in this case, no care was taken about lighting during the whole procedure.

Results

Formation of photodecomposition products

Figure 1 shows gas chromatograms for photodecomposition process of nifedipine, nisoldipine and nitrendipine; the authentic compounds, 5 μg each in methanol, had been photodecomposed under a fluorescent lamp (400—600 nm, 4000 lux) for 0, 10 and 60 min.

Even at 0 min, very small peaks of intermediates in photodecomposition appeared which are marked with α ,β , σ (Fig.1, left panel).

After exposure for 60 min, nifedipine and nisoldipine disappeared, and the final products (a and b) appeared, which were relatively stable for at least 2 h of exposure. However, for nitrendipine, such a final product did not appear; a slightly smaller peak of nitrendipine itself and its growing intermediate (σ) were observed (Fig.1, right panel).

Positive and negative mass spectra of the unchanged forms of drugs

PIEI, PICI and NICI mass spectra of nifedipine, nisoldipine and nitrendipine together with each probable fragmentation mode, are shown in Figs.2—4.

In the PIEI mode, molecular cations were generally small. Nifedipine and nisoldipine gave

intense $[M\text{-}OH]^+$ cations, which constituted base peaks. For nitrendipine, a cation at m/z 238, which may be due to loss of nitrophenyl group from the molecule, constituted the base peak.

In the PICI mode, all three drugs showed $[M+1]^+$ quasi-molecular peaks together with $[M+C_2H_5]^+$ ions. For both nifedipine and nitrendipine, the ions at m/z 315 showed the base peaks, which are due to loss of methoxy and ethoxy groups from the molecules, respectively.

In the NICI mode, all drugs showed intense M^- anions, which constituted base peaks, and only a few fragment peaks.

Positive and negative mass spectra of the photodecomposition products of nifedipine and nisoldipine

PIEI, PICI and NICI mass spectra of the final photodecomposition products (a and b) from nifedipine and nisoldipine are shown in Figs.5 and 6. The spectra of intermediate products (α, β and σ) were not measured because of their instability.

By careful measurements of each mass spectrum in the three modes and analysis of the spectra, the products (a and b) were estimated to be dehydronitrosonifedipine and dehydronitrosonisoldipine, respectively.

In the PIEI mode, the base peaks of both compounds were due to loss of each side chain attached to the pyridyl ring.

In the PICI mode, intense $[M+1]^+$ quasi-molecular peaks appeared for both compounds together with $[M+CH_3]^+$, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ ions.

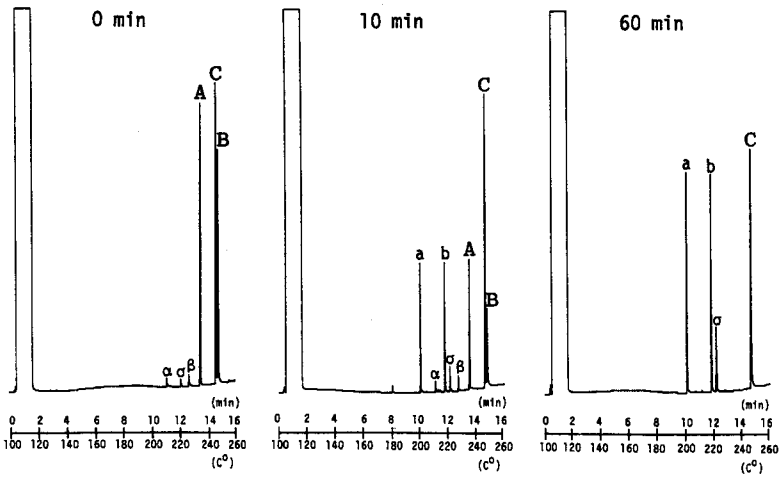

Fig. 1. Capillary GC showing the photodecomposition of the authentic nifedipine, nisoldipine and nitrendipine in methanol solution at different times of their exposure to a fluorescent lamp (400−600 nm, 4000 lux). A, nifedipine; B, nisoldipine; C, nitrendipine; α, β and σ, intermediate from A, B and C, respectively; a and b, the final products from A and B, respectively.

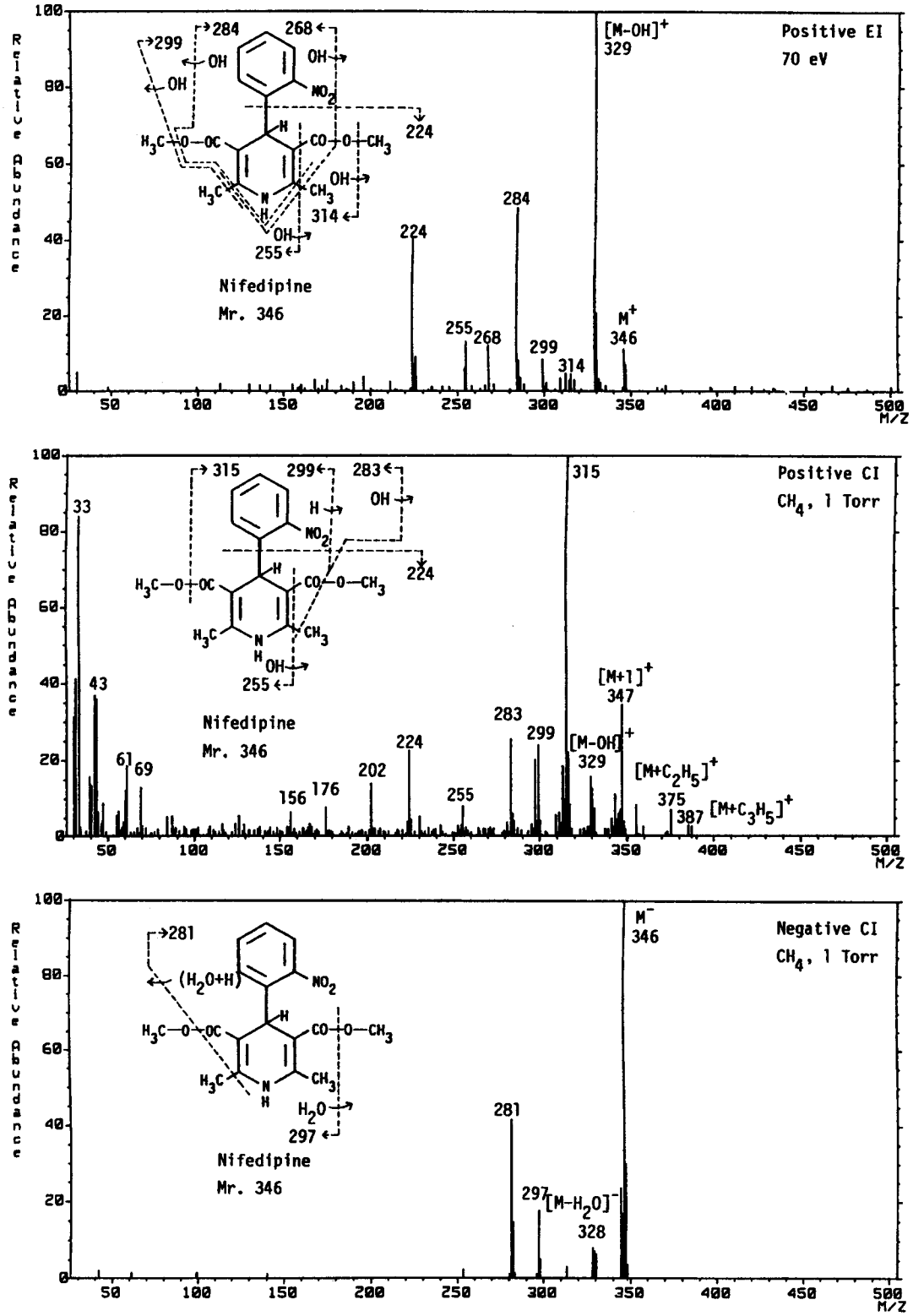

Fig. 2. PIEI, PICI and NICI mass spectra of nifedipine and its probable fragmentation modes.

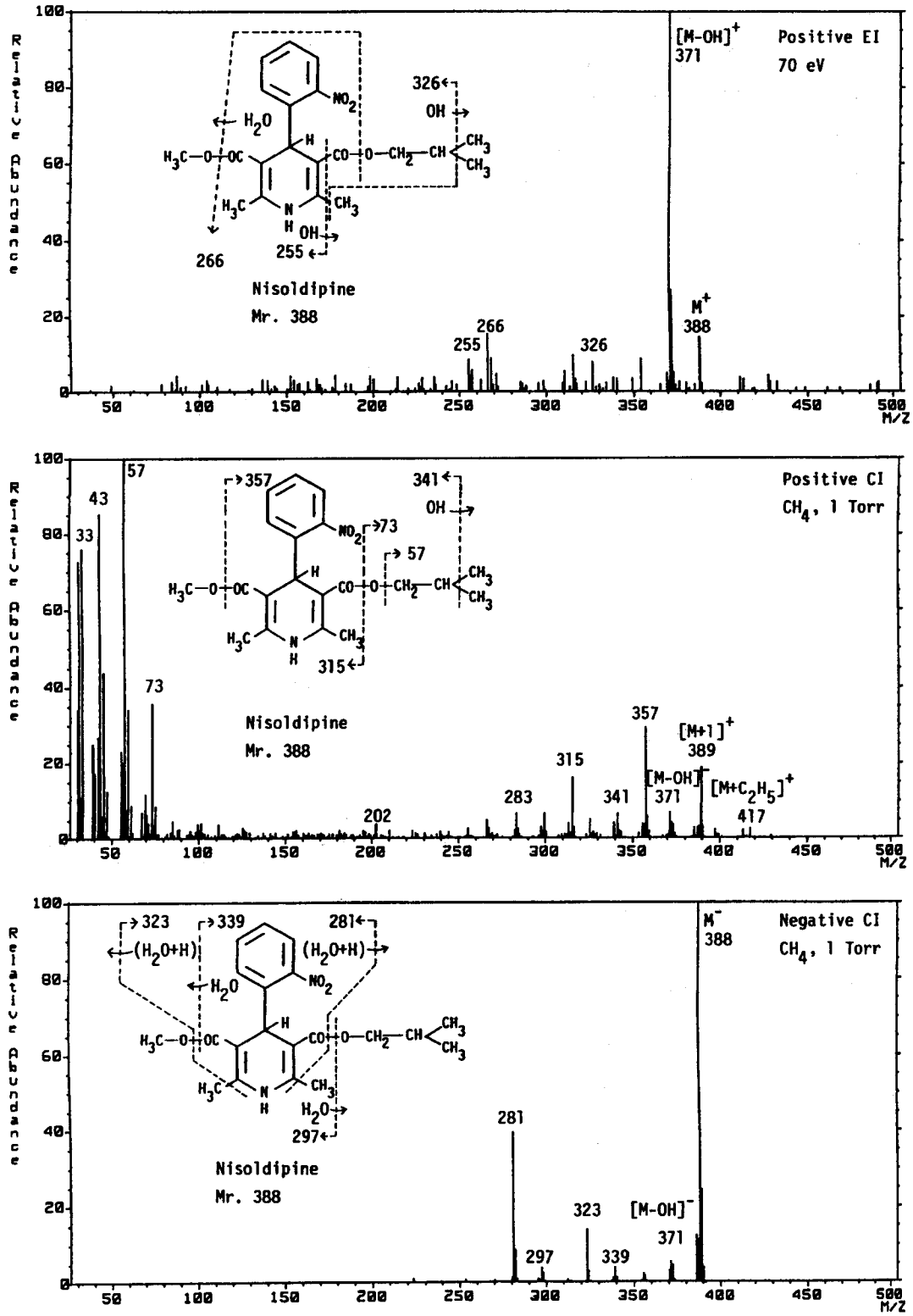

Fig. 3. PIEI, PICI and NICI mass spectra of nisoldipine and its probable fragmentation modes.

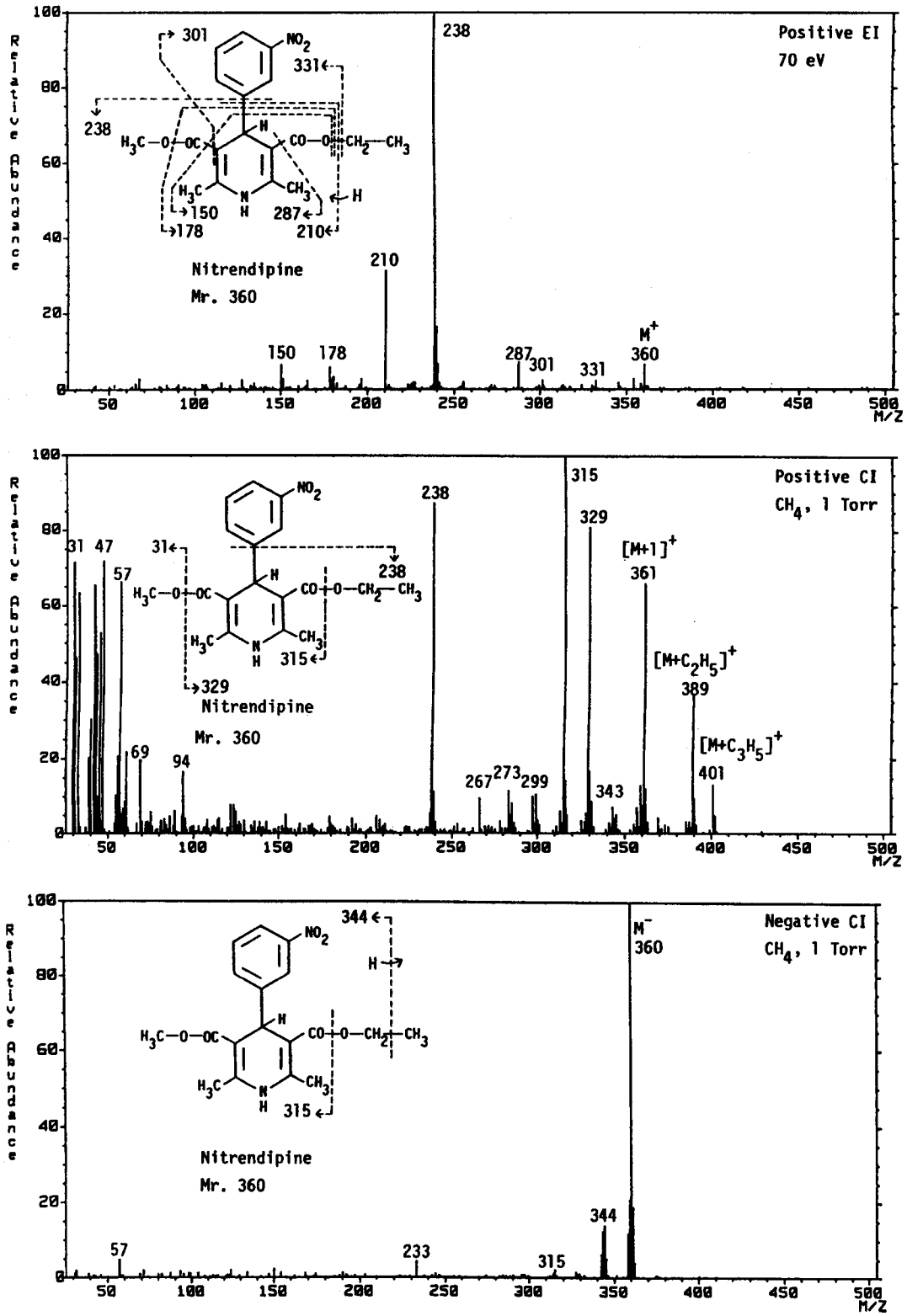

Fig. 4 . PIEI, PICI and NICI mass spectra of nitrendipine and its probable fragmentation modes.

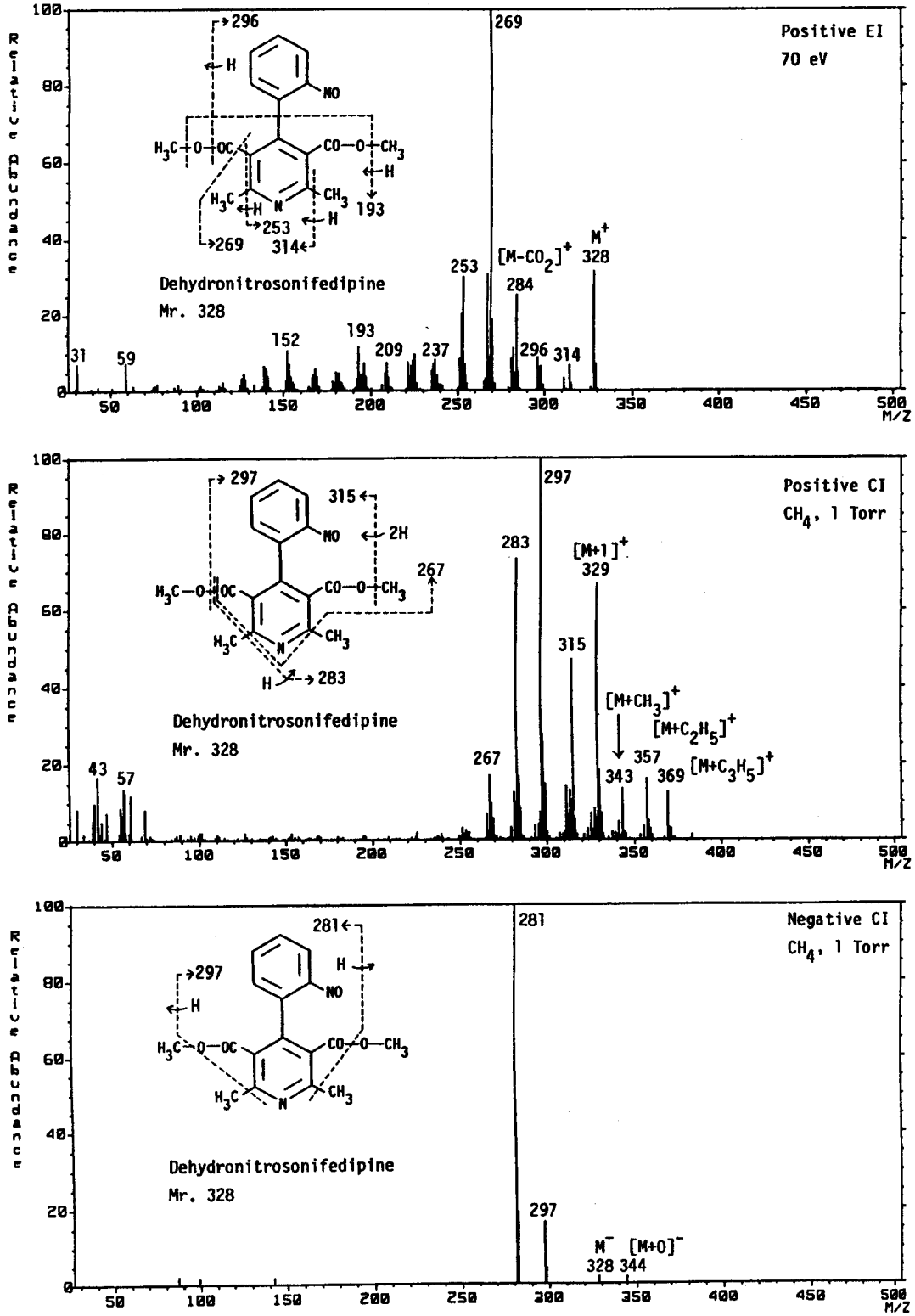

Fig. 5 . PIEI, PICI and NICI mass spectra of the photodecomposition product of nifedipine (dehydronitrosonifedipine) and its probable fragmentation modes.

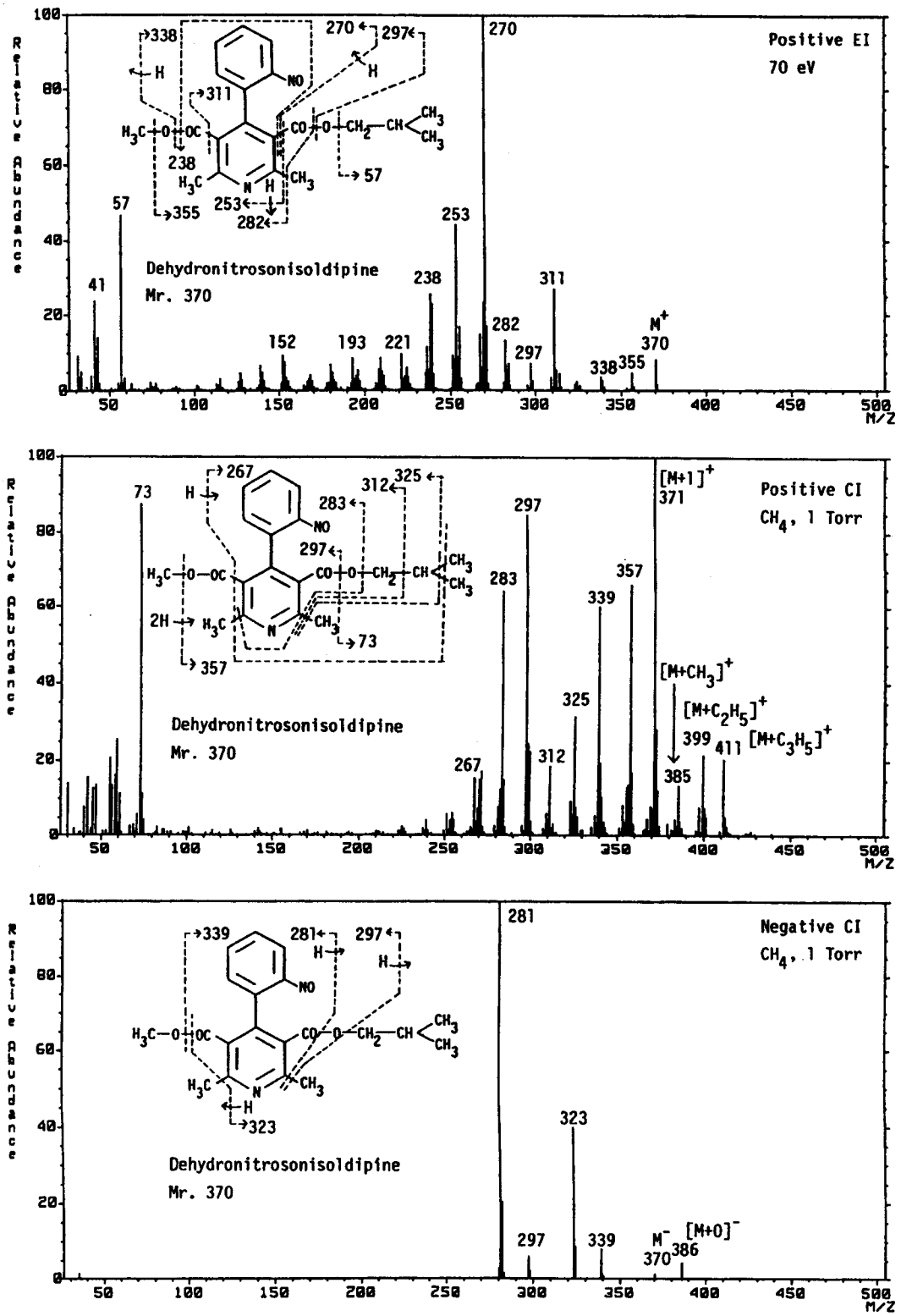

Fig. 6. PIEI, PICI and NICI mass spectra of the photodecomposition product of nisoldipine (dehydronitrosonisoldipine) and its probable fragmentation modes.

In the NICI mode, molecular anions were very small for both compounds; small $[M+O]^-$ adduct anions appeared. Fragment anions at m/z 281 constituted base peaks for both compounds.

Sensitivity by total ion monitoring (TIM) in different modes

To check sensitivity of the present GC/MS method, intensities of peaks, obtained by TIM with the DB-1 capillary column, were compared with each other in the three modes. The detection limits in the PIEI and NICI mode were 1−10 ng for nifedipine, nisoldipine and nitrendipine, and 1−8 ng for dehydronitrosonifedipine and dehydronitrosonisoldipne in an injected volume. In the PICI mode, they were 8−10 ng for nifedipine, nisoldipne and nitrendipine, and 30−40 ng for dehydronitrosonifedipine and dehydronitrosonisoldipine in an injected volume.

Clean-up and capillary GC

To enable actual identification in human samples by GC/MS, a mixture of the three drugs, 5 μg of each, was added to 1 ml of whole blood, serum or urine, extracted with Sep-Pak C_{18} cartridges in a dark room to avoid photodecomposition and detected by capillary GC. The gas chromatograms obtained with the DB-1 column are shown in Fig.8. Nifedipine, nisoldipine and nitrendipine could be satisfactorily separated from each other and from impurities on the gas chromatograms. Recovery of the drugs from the whole blood, serum and urine was more than 79 %, 90 % and 98 %, respectively.

Figure 8 shows gas chromatograms obtained from the same experiments as above, except that no care was taken for avoiding photodecomposition (all experiments were done in a light room) and also that, after extraction, the residue dissolved in methanol was exposed to a fluorescent lamp for complete photodecomposition. The peaks due to dehydronitrosonifedipine (a), dehydronitrosonisoldipine (b) and unchanged nitrendipine (C) appeared. They could be satisfactorily separated from each other and from impurities on gas chromatograms. The recovery of dehydronitrosonifidipine, dehydronitrosonisoldipine and nitrendipine were 84 %, 80 % and 100 %, respectively.

Discussion

In this paper, we have presented PIEI, PICI and NICI mass spectra of nifedipine, nisoldipine, nitrendipine, dehydronitrosonifedipine and dehydronitrosonisoldipine. Such systematic studies in the three modes have never been reported to our knowledge.

The NICI mode MS is most powerful to detect a nitro group in a chemicals [7]. In this

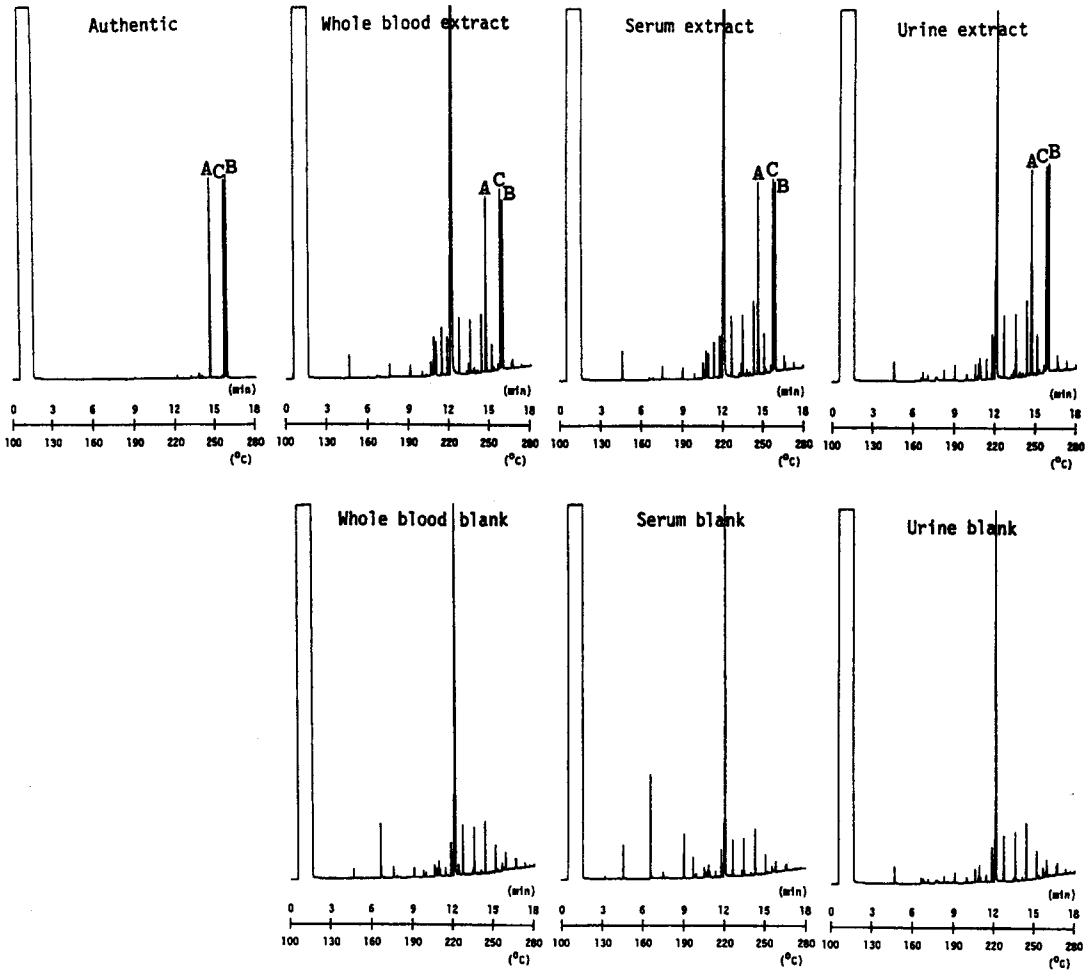

Fig. 7 . Capillary GC for nifedipine (A), nitrendipine (C) and nisoldipine (B) isolated from human whole blood, serum and urine by use of Sep-Pak C_{18} cartridges. The mixture of three drugs (5 μg each) was added to 1 ml of each sample. The extraction procedure was carried out in a dark room to prevent the drugs from photodecomposition.

study, nifedipine, nisoldipine and nitrendipine, which had nitro groups in their structures, gave an intense M^- peak and constituted base peaks (Figs.2−4). For dehydronitrosonifedipine and dehydronitrosonisoldipine, which had a nitroso group in their structures, intense anion at m/z 281 appeared and constituted base peaks (Figs. 5 and 6). These base peaks are ideal for specific and sensitive quantitative MS analysis of these drugs in both unchanged and photodecomposed forms in the NICI mode.

This study is also the first trial for using Sep-Pak C_{18} cartridges for the clean-up of nifedipine, nisoldipine and nitrendipine from biological samples. In the previous reports, these drugs were usually isolated by liquid-liquid extraction [6, 8−10]. The advantages of the

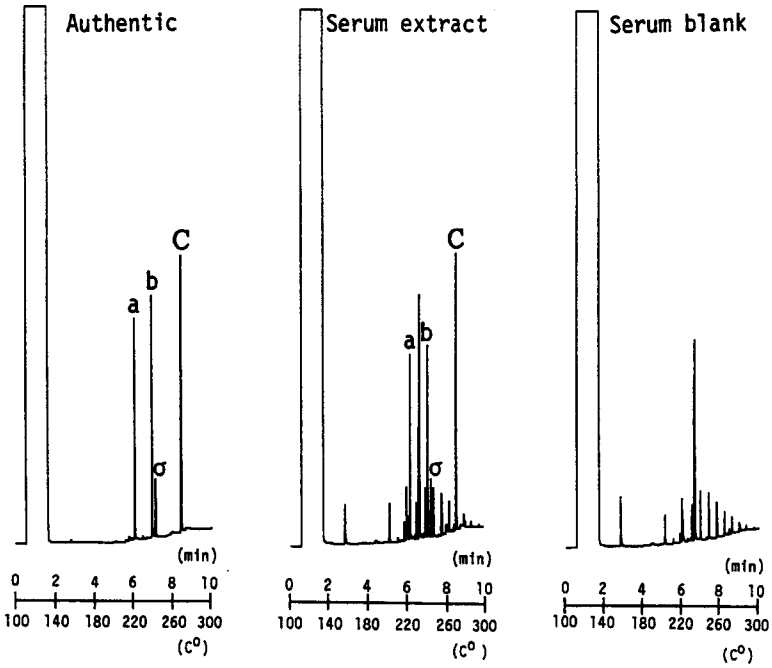

Fig. 8. Capillary GC for a serum sample, to which nifedipine, nisoldipine and nitrendipine had been added after extraction with Sep-Pak C_{18} cartridges and photodecomposition under a fluorescent lamp for 60 min. The mixture of three parent drugs (5 μg each) was added to 1 ml of serum. All procedure was carried out in a light laboratory. a, dehydronitrosonifedipine; b, dehydronitrosonisoldipine; σ, dehydronitrendipine; C, nitrendipine.

Sep-Pak cartridges are that the analytical procedure is much simpler and faster than that with organic solvents, and that much cleaner extracts can be obtained.

It was reported that nifedipine and nisoldipine were very sensitive to photodecomposition and easily dehydrated to yield dehydronitrosopyridinyl compounds *via* dehydrogenation; nitrendipine were only dehydrogenated to dehydronitrendipine by light [5]. The extent of the photodecomposition of nifedipine and nisoldipine was dependent upon light intensity [11]. These results have been confirmed in the present study (Figs.1, 5 and 6).Thus, the intermediate compounds (α, β and σ) found in the gas chromatograms during photodecomposition (Fig.1) seem to be dehydrogenated forms (M-2H) of each compound. The difference in the photosensitivity found between nifedipine/nisoldipine and nitrendipine is due to the *ortho* and *meta* positions of the nitro groups; the oxygen atom of the *ortho*-nitro group is easily coupled with two hydrogen atoms of the pyridyl ring, but not that of the *meta*-nitro group [5].

The present paper is composed of mass spectra of nifedipine, nisoldipine, nitrendipine, dehydronitrosonifedipine and dehydronitrosonisoldipine in different modes, a solid-phase extraction method with and without photodecomposition, and capillary GC. Our data give useful

informations on detection and identification of the three calcium antagonists in both unchanged and photodecomposed forms from biological samples by both GC and GC/MS. We recommend the procedure for the drugs with photodecomposition, because no special care about lighting of the laboratory is required during the whole procedure of analysis.

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