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Note

Gas chromatographic determination of nifedipine and one of its metabolites using electron capture detection

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Nifedipine, 4-(2'-nitrophenyl)-2,6-dimethyl-3,5-dicarbomethoxy-1,4-dihydropyridine, BAY a 1040, is a calcium antagonistic drug with a marked effect on the excitation—contraction coupling in different types of smooth muscle and myocardium [1,2].

Clinical studies have demonstrated that nifedipine is an effective drug in the treatment of coronary heart disease, and preliminary results in the treatment of arterial hypertension have been promising [3].

The finding of a close correlation between the clinical effects and the plasma concentrations of nifedipine [3], measured by a relatively troublesome and time consuming fluorimetric method [4], has called for the development of a sensitive and specific gas chromatographic (GC) method for determination of nifedipine in blood plasma.

EXPERIMENTAL

Standards and reagents

Nifedipine as a pure crystalline compound and in solution for injection (0.1 mg/ml) was kindly supplied by Bayer, Leverkusen, G.F.R. Standard solutions of nifedipine were protected from light and stored at 4°.

Toluene (analytical reagent) and tritisol buffer (pH 9) were obtained from Merck, Darmstadt, G.F.R.

The internal standard solution was 1 μg/ml of diazepam in water.

Preparation of the metabolite

A 1-ml sample of nifedipine (0.1 mg/ml) was oxidized at 50° for 1 h with 2 ml 0.01 *M* potassium permanganate at pH 9. After cooling to room tempera-

ture the aqueous solution was extracted three times with 10 ml of diethyl ether. After evaporation at 30° with a gentle stream of nitrogen, the residue was dissolved in a suitable amount of ethanol. A standard solution of oxidized nifedipine corresponding to 10 μg/ml of nifedipine in ethanol was prepared.

Apparatus

A Varian 2100 gas chromatograph equipped with a [63] Ni electron capture detector (DC mode) was used. The GC column was glass (180 cm × 2 mm I.D.) filled with 2% OV-17 (Pierce, Rockford, Ill., U.S.A.) on Gas Chrom Q, 80—100 mesh, conditioned for 24 h at 275° with a nitrogen flow-rate of 25 ml/min. The operating conditions were: column temperature 240°, injector temperature 240°, detector temperature 290° and carrier gas (nitrogen) flow-rate 25 ml/min. Under these conditions the metabolite, the internal standard (diazepam) and nifedipine had retention times of 1.3, 2.4 and 4.0 min respectively.

For the mass spectrometric work a Jeol D-100 mass spectrometer connected to a gas chromatograph was used. Conditions: ionization voltage 25 eV, ionization current 300 μA. The separator (double stage jet) was heated to 260°. The GC column was a 1 m, 3% OV-17 on Gas-Chrom Q, 80—100 mesh., column temperature 220° and carrier gas (helium) 20 ml/min.

Procedure

To a 10-ml glass-stoppered centrifuge tube containing 1 ml of tritisol buffer (pH 9), 250 μl of the internal standard solution (1 μg/ml diazepam in water)
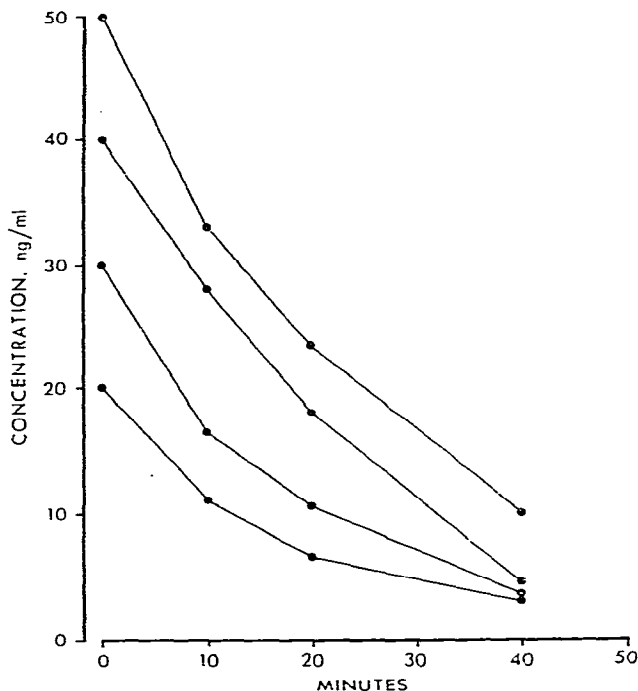

Fig. 1. Degradation of nifedipine in 4 different concentrations after exposure to normal laboratory light. Solvent: toluene.
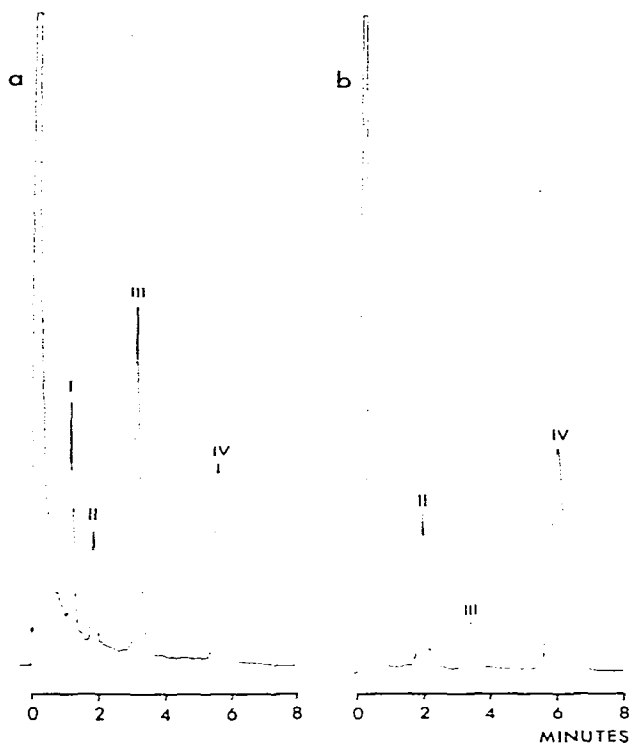
Fig. 2. Gas chromatograms of the light-degradation product (I), the oxidation product (II), diazepam (III) and nifedipine (IV). (a) Injection of approx. 100 ng of each compound using flame ionization detection; solvent: chloroform; (b) injection of approx. 100 pg of each compound using electron capture detection; solvent: toluene. Column temperature: 220°.

and 1 ml of toluene was added 0.5 ml of a plasma sample. The tube was shaken vigorously on a Whirly mixer for 15 sec. After centrifugation (3000 rpm, 10 min) 2 µl of the organic phase was injected onto the gas chromatograph. During the whole procedure the centrifuge tube was protected from light.

Preparation of standard curves

Known amounts of nifedipine and the synthetized metabolite were added to human plasma. Samples of 0.5 ml were treated as described above. The standard curves were constructed by plotting the ratio of the peak height of nifedipine or metabolite and that of the internal standard (diazepam) against the concentration of nifedipine or metabolite.

RESULTS AND DISCUSSION

Nifedipine is sensitive to light. Fig. 1 shows the degradation of nifedipine in toluene solution at different concentrations after exposure to normal laboratory light. Logarithmic transformation of the data revealed a first order kinetics with a rate constant of 0.05 min^{-1} for the degradation. In the dark nifedipine is stable in toluene for at least one day. Consequently care was taken to protect the samples from day light. Heparinized plasma samples were stored in brown

centrifuge tubes and during the extraction procedure the samples were kept in cardboard containers.

Oxidation of nifedipine under mild conditions gives a product which has excellent GC and electron capturing properties. This oxidation product is resistant to light exposure; therefore conversion of nifedipine by oxidation might seem to be a possible method for determination of nifedipine. However, plasma samples from individuals receiving the drug show a GC peak with exactly the same retention time as that of the oxidation product. This finding suggests that the oxidized drug is a metabolite of nifedipine.

While nifedipine and its oxidized derivative have excellent electron capturing abilities the opposite is found for the light-degradation product. Fig. 2 shows gas chromatograms of the three compounds and the internal standard using flame ionization and electron capture detection.

In order to obtain optimal extraction conditions 0.5 ml amounts of plasma samples containing 100 ng/ml of nifedipine were adjusted to different pH values. 100% extraction was accomplished at pH values between 3 and 13 with equal amounts of organic and aqueous phase using an extraction time of 15 sec.

Fig. 3 shows gas chromatograms of plasma samples after extraction. As shown in Fig. 4 the standard curves for nifedipine and the oxidation product were linear between 0 and 100 ng/ml with regression coefficients of 0.9983
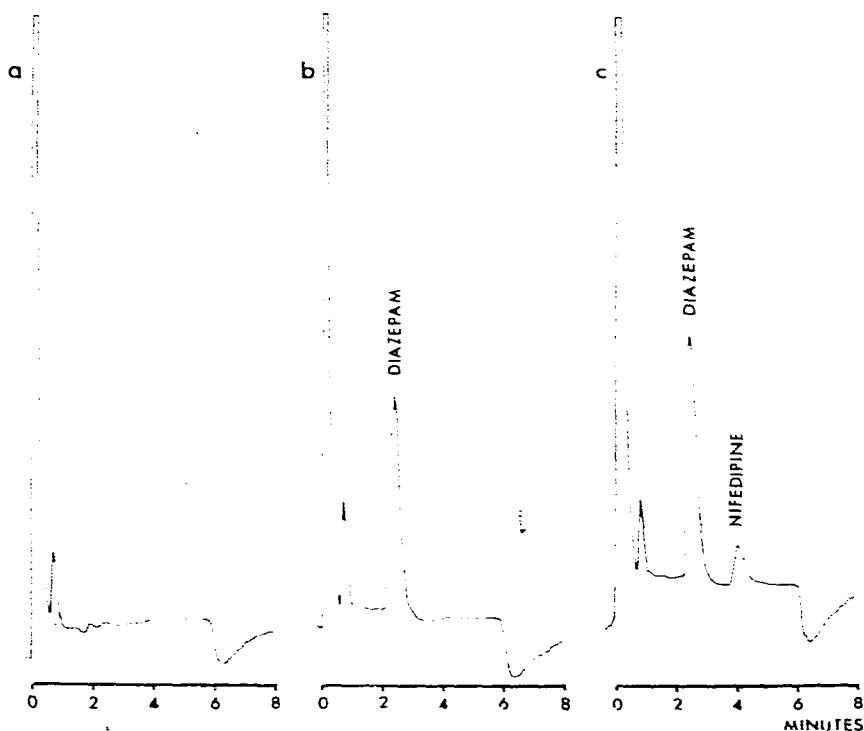

Fig. 3. Gas chromatograms of human plasma samples after toluene extraction. (a) Plasma blank; (b) after addition of diazepam, 500 ng/ml; (c) after addition of diazepam, 500 ng/ml, and nifedipine, 25 ng/ml. Column temperature: 240°.
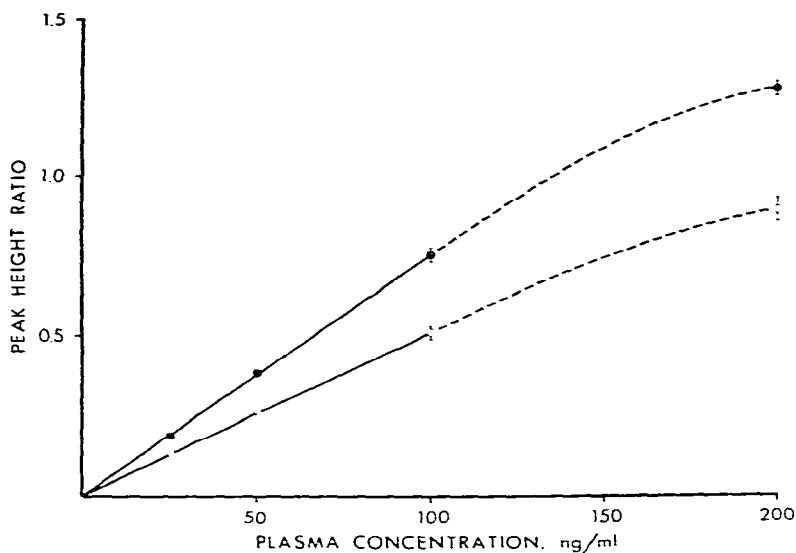
Fig. 4. Standard curves for determination of plasma concentrations of nifedipine, ● (r = 0.9983, 0—100 ng/ml) and the metabolite, ○ (r = 0.9976, 0—100 ng/ml). Each point is the mean(± S.D.) of the results from 6 determinations. The ordinate refers to the ratio between the peak height of nifedipine or metabolite and the peak height of diazepam.
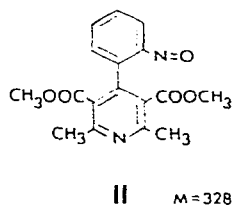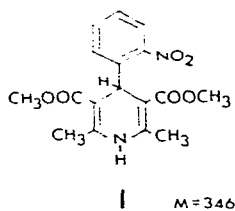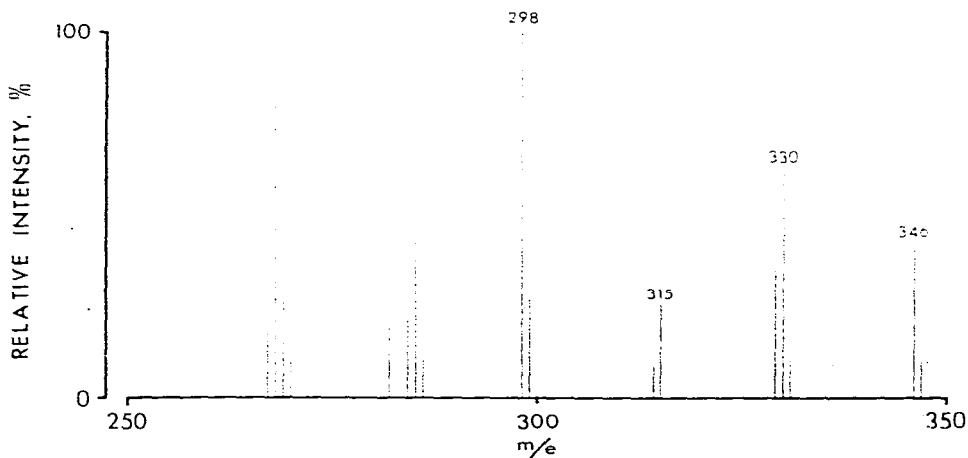

Fig. 5. Mass spectrum of nifedipine and structural formulae of nifedipine (I) and the light-degradation product (II).

for nifedipine and 0.9976 for the oxidation product. The minimum detectable concentration was found to be about 1 ng/ml for both nifedipine and the metabolite.

Within-run precision was determined from 6 separate extractions of plasma samples containing 50 and 100 ng of nifedipine or metabolite per ml of plasma. The within-run coefficient of variation was 2.1% for 50 ng/ml and 2.8% for 100 ng/ml with respect to nifedipine. The coefficient of variation for determination of the metabolite was 3.5% for 50 ng/ml and the same for 100 ng/ml.
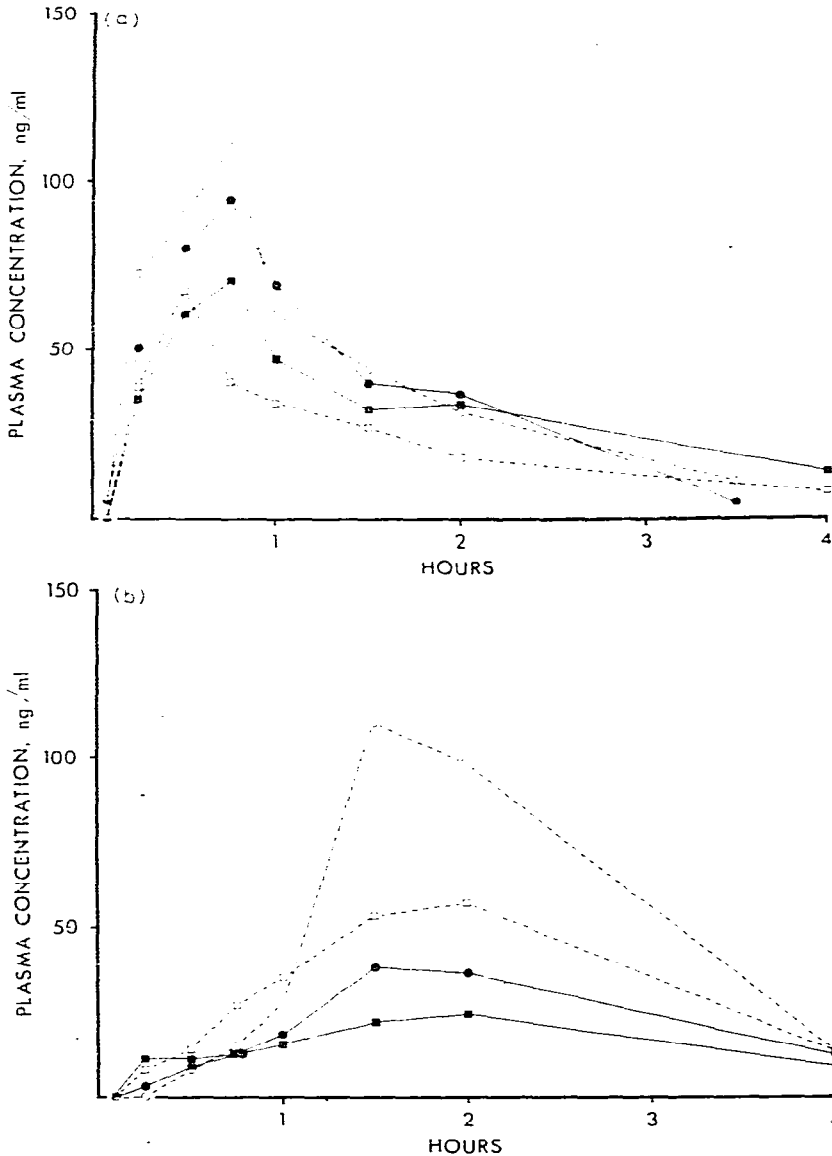

Fig. 6. Plasma concentrations of nifedipine (solid line) and the metabolite (broken line) as a function of time in four healthy persons after sublingual administration of 10 mg of nifedipine. (a) Two persons showing fast absorption; (b) two persons showing slow absorption.

Combined GC—mass spectrometry (GC—MS) confirms, that nifedipine is stable on the GC column. Fig. 5 shows the GC—MS of nifedipine (I). The GC—MS of the light-degradation product showed a molecular ion 18 mass units smaller than that of nifedipine, consistent with formula II. The GC—MS of the oxidation product showed an extremely large base peak at *m/e* 298 and a small ion at *m/e* 313. It is likely that nifedipine is easily oxidized in the dihydropyridine ring to the pyridine derivative. Using the fluorimetric method [4] both compounds are determined as nifedipine. In preparations of isolated human veins this metabolite is ca. one hundred times less potent than nifedipine in inhibiting the potassium- and noradrenaline-induced contraction. The structure of the metabolite has not yet been confirmed.

APPLICATION

Sublingual administration of 10 mg of nifedipine was performed in four healthy volunteers. They were instructed to bite the capsule apart and keep the substance in the mouth for 5 min before swallowing. Blood samples were drawn 0, 5, 15, 30, 45, 60, 120, 240 and 480 min after administration. After immediate centrifugation the plasma was stored at -20° until analysis was carried out. Plasma concentrations of nifedipine as well as the metabolite were determined. Two types of absorption curves were found. Fig. 6 shows the plasma concentration curves from the four persons. Two persons had a fast absorption with maximum concentrations of nifedipine of 70—100 ng/ml reached within 30—60 min after administration (Fig. 6a) and two persons had a slower absorption with maximum concentrations of 20—40 ng/ml reached within 90—120 min after administration (Fig. 6b).

Further studies on the pharmacokinetics and metabolism of nifedipine are in progress.

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