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DETERMINATION OF NIFEDIPINE IN HUMAN PLASMA BY CAPILLARY GAS CHROMATOGRAPHY WITH NITROGEN DETECTION

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SUMMARY

A gas chromatographic method for the determination of nifedipine in human plasma is presented. Nifedipine was extracted from plasma at basic pH with toluene, and nitrendipine was used as the internal standard. Chromatography was performed on a cross-linked methylsilicone fused-silica column by on-column injection and with a nitrogen–phosphorus ionization detector. The minimal detectable concentration was ≈ 0.5 ng/ml of plasma. The standard curve was linear in the range evaluated, 2–300 ng/ml plasma. The within-analysis coefficient of variation was 3.9–10.4%, and the day-to-day coefficient of variation was 3.8%. A peak with the same retention time as the nitropyridine derivative of nifedipine was detected in the plasma of patients who had taken 10 mg nifedipine orally.

INTRODUCTION

The calcium antagonist nifedipine (dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate) (Adalat®) elicits a number of cardiovascular effects and is used, *e.g.*, in treatment of angina pectoris and hypertension[1]. Assay methods have been developed for the measurement of unchanged nifedipine, alone or together with its metabolites; these assays are complicated by the light sensitivity of the product[2].

A fluorometric method, developed by Schlossmann[3], is not specific. Other methods for the determination of nifedipine in human plasma are selected-ion monitoring[4] and gas chromatography (GC) with electron capture detection[5–8]. High-performance liquid chromatography[9] and GC with flame ionization detection[10] were used for the determination of nifedipine in rat plasma. In some of these methods nifedipine is oxidized to its more stable nitropyridine derivative[5,7,8,10].

The combination of on-column injection and a cross-linked fused-silica column in capillary GC has recently become available as a powerful technique for the assay of compounds that pose difficulties in classical GC analysis. We report here a method for the determination of nifedipine in human plasma, based on this technique, with nitrogen-selective detection and the use of the analogue nitrendipine as internal standard. The method also allows detection of two possible metabolites of nifedipine. As

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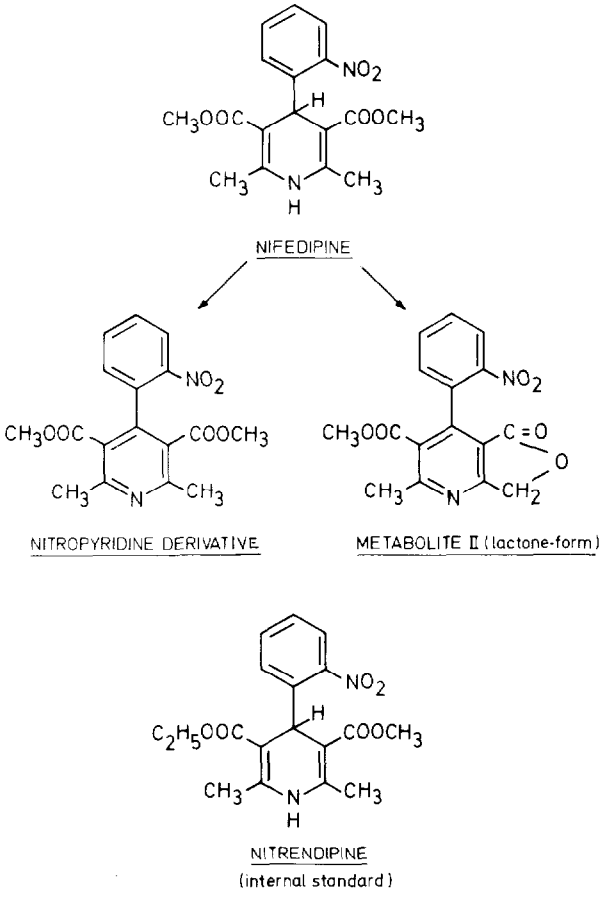

Fig. 1. Structure of nifedipine, of two of its metabolites and of the internal standard, nitrendipine.

an application of the method, gas chromatograms obtained from a patient receiving nifedipine are shown.

EXPERIMENTAL

Chemicals

Nifedipine (BAY a 1040), the internal standard nitrendipine (BAY e 5009) and two possible metabolites, the nitropyridine derivative of nifedipine and BAY h 2228 (mentioned in the literature as metabolite II) were gifts from Bayer (Wuppertal, F.R.G.). The chemical structures are shown in Fig. 1.

Toluene was pesticide grade (Carlo Erba, Milan, Italy), all other chemicals were of analytical grade.

Gas chromatography

A Hewlett-Packard gas chromatograph Model 5880 equipped with a nitrogen–phosphorus ionization detector was used. GC was performed on a 12 m

× 0.31 mm I.D. cross-linked methylsilicone fused-silica column, film thickness 0.52 μm, with a helium flow of 6 ml/min. A Hewlett-Packard capillary on-column injection port system (19320 H) was used. Samples were injected with a Hamilton 10-μl syringe and a fused-silica needle (0.18 mm O.D.). Temperatures: injector, 230°C; oven, 210°C; detector, 310°C.

Detector conditions were set on a high response: helium make-up gas, 20 ml/min; hydrogen, 3.5 ml/min; air, 75 ml/min.

The column had to be washed with methanol after 1 month of daily use.

Extraction procedure

A 7-μl aliquot of an internal standard solution, containing 70 ng of nitrendipine, was added to 1 ml plasma in a 10-ml glass-stoppered brown centrifuge-tube, and the plasma was brought to pH 9 with 0.1 *M* sodium hydroxide solution. The mixture was extracted with 5 ml of toluene by shaking it horizontally for 20 min. After centrifugation for 10 min at 4300 *g*, the organic phase was transferred with a Pasteur pipette to a 6-ml glass-stoppered brown conical tube, and the organic phase was evaporated under a gentle stream of nitrogen at 40°C. Another 4 ml of toluene were added to the aqueous phase, which was shaken and centrifuged as before. The toluene layer was then transferred to the same conical tube, and again evaporated under nitrogen. Before evaporation was complete, the wall of the conical tube was washed down with 300 μl of toluene, and the toluene was evaporated to dryness. The

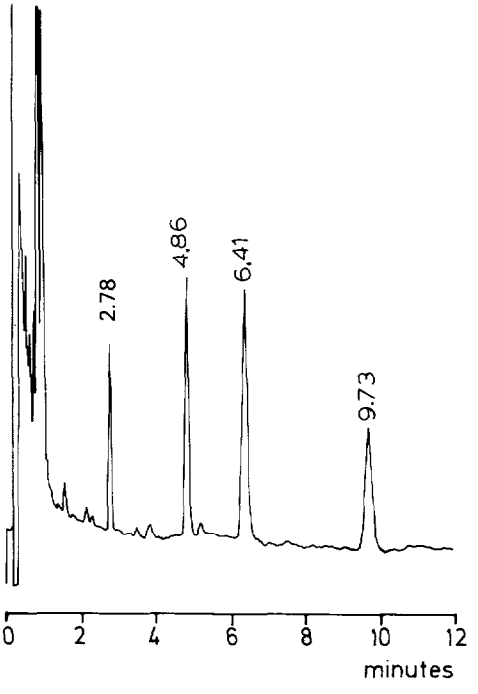

Fig. 2. GC chromatogram of a 1-ml plasma extract. Blank plasma, spiked with 50 ng/ml of the nitropyridine metabolite (t_R 2.78 min), 100 ng/ml of metbolite II (t_R 4.86 min), 100 ng/ml of nifedipine (t_R 6.41 min) and 70 ng/ml of the internal standard nitrendipine (t_R 9.73 min).

TABLE I

EFFICIENCY OF THE EXTRACTION RECOVERY FOR NIFEDIPINE

Concentration (ng/ml)	*Recovery (%)*
2	93.1
5	96.3
10	86.9
20	95.8
40	96.3

residue was reconstituted in 10 μl of toluene, and 0.7 μl were injected into the chromatograph. The whole procedure was carried out under subdued light.

RESULTS AND DISCUSSION

Under the chromatographic conditions used, nifedipine, the nitropyridine derivative of nifedipine, metabolite II and nitrendipine gave fully resolved, essentially symmetrical peaks, eluted in 10 min (Fig. 2). The efficiency of the extraction recovery for different concentrations of nifedipine is given in Table I. Over the 2–300 ng/ml plasma range studied, the standard curve is linear. The standard curves analysed over 1 month ($n = 10$) gave a mean regression ($\pm$S.D.) of $y = -0.0187\ (\pm\ 0.0431) + 0.0231\ (\pm\ 0.0030)\ x$ with $r = 0.9949\ (\pm\ 0.0040)$, where y is the nifedipine peak height divided by the nitrendipine peak height and x is the concentration (ng/ml) of nifedipine. The within-analysis precision and accuracy are given in Table II.

The reproducibility of the method was demonstrated by a day-to-day coefficient of variation (C.V.) of 3.8% ($n = 12$) using pooled plasma, spiked with nifedipine (100 ng/ml), and analysed over 1 month. The mean assay result was 98.9%. These plasma samples were kept for 4 months at -20°C before being processed, proving the stability of the product under these conditions.

The detection limit of this assay with the described instrumentation and procedure was $\approx$0.5 ng/ml.

The following drugs, when injected directly in amounts expected from patients given therapeutic concentrations, did not interfere with the assay: propranolol, aten-

TABLE II

WITHIN-RUN ACCURACY AND PRECISION FOR NIFEDIPINE ($n = 5$)

Concentration (ng/ml)	*Mean result (%)*	*C.V. (%)*
5	105.8	10.4
10	99.2	5.4
20	99.8	6.7
40	105.0	5.5
60	99.3	3.9
80	104.7	4.8

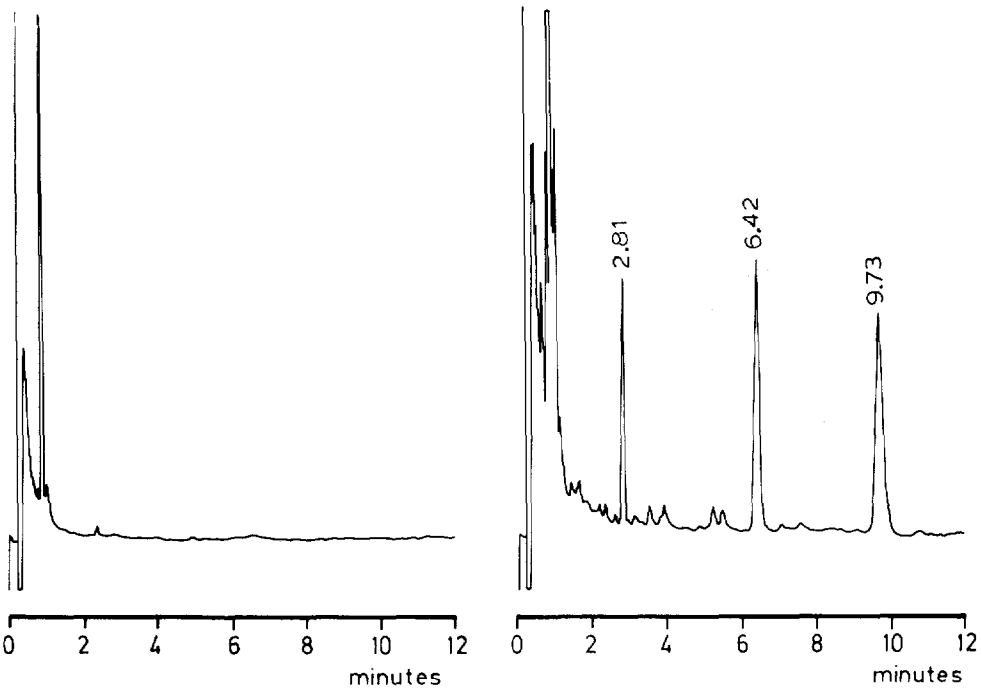

Fig. 3. GC chromatograms of a 1-ml plasma extract: left, blank plasma of a patient; right, plasma of the same patient 1 h after oral intake of 10 mg of nifedipine (t_R 6.42 min). The latter was spiked with 70 ng/ml of the internal standard (t_R 9.73 min). The peak at 2.81 min is probably the nitropyridine metabolite of nifedipine.

olol, metoprolol, digoxine, disopyramide, lidocaine and procainamide. However, interference of quinidine can occur, as its retention time (t_R, 6.26 min) is close to that of nifedipine. Aprindine (t_R 4.8 min) could interfere with metabolite II.

Chromatograms of plasma obtained from a patient before and 1 h after oral administration of 10 mg of nifedipine are shown in Fig. 3. The blank plasma yields no interfering peaks from endogenous components. Nifedipine (t_R 6.42 min) is present in the plasma of the patient 1 h after intake of 10 mg nifedipine. A peak (t_R 2.81 min) with about the same retention time as the nitropyridine derivative of nifedipine is found. This peak was also seen by others after oral[7,8] and sublingual[5] administration of nifedipine, but not after intravenous administration[8]. However, the identity of this substance with the nitropyridine derivative has not been proven. No detectable amounts of metabolite II are present.

The described GC assay allows specific and quantitative determination of nifedipine in plasma. By using a cross-linked phase in a fused-silica column in conjunction with a nitrogen–phosphorus detector, subnanogram amounts of nifedipine can be detected. The use of this detector permits the clean-up procedure to be less extensive than with the use of an electron-capture detector[7].

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