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Capillary gas chromatographic determination with nitrogen–phosphorus detection of the calcium antagonist nicardipine and its pyridine metabolite M-5 in plasma

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Abstract

A capillary gas chromatographic method with nitrogen–phosphorus detection for the simultaneous determination of nicardipine and its pyridine metabolite M-5 was developed. The method involves extraction of the plasma with hexane–methylene chloride (1:1, v/v), followed by evaporation of the organic phase. The extract is injected into a fused-silica capillary column coated with cross-linked 5% phenyl–methylsilicone. A temperature gradient (85–285°C) is applied and the two products and the internal standard can be separated within 22 min. The limit of detection is 0.5 ng/ml for both products. The method is suitable for pharmacokinetic studies in humans.

1. Introduction

Nicardipine [2(N-methylbenzylamino)ethyl-methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-diarboxylate hydrochloride] is a dihydropyridine calcium channel blocker with coronary and peripheral arterial vasodilatory activity used for the treatment of angina pectoris and hypertension [1]. Studies in animals and humans of the metabolism and pharmacokinetics of orally administered nicardipine demonstrated that it was rapidly absorbed, extensively first-pass metabolized to several metabolites (Fig. 1) and rapidly eliminated [1]. The degradation of nicardipine measured in aqueous solution suggests that nicardipine hydrochloride, although light sensitive, is less dramatically modified than nifedipine [2].

Plasma levels of nicardipine have been determined by packed column gas chromatography (GC) with electron-capture detection (ECD) [3]. Using this method, nicardipine was determined following oxidation to a pyridine analogue metabolite [M-5]. A laborious thin-layer chromatographic–GC–mass spectrometric method for the determination of the individual concentrations of nicardipine and its pyridine metabolite has been described [4]. Reported HPLC methods are not sensitive enough [5–7] as the detection limits are >2 ng/ml. Wu *et al.* [8] used a capillary GC method with ECD but the calibration graphs were not linear.

This paper describes a specific and sensitive capillary GC (cGC) method for the simultaneous determination of nicardipine and its metabolite M-5 in the plasma of healthy volunteers.

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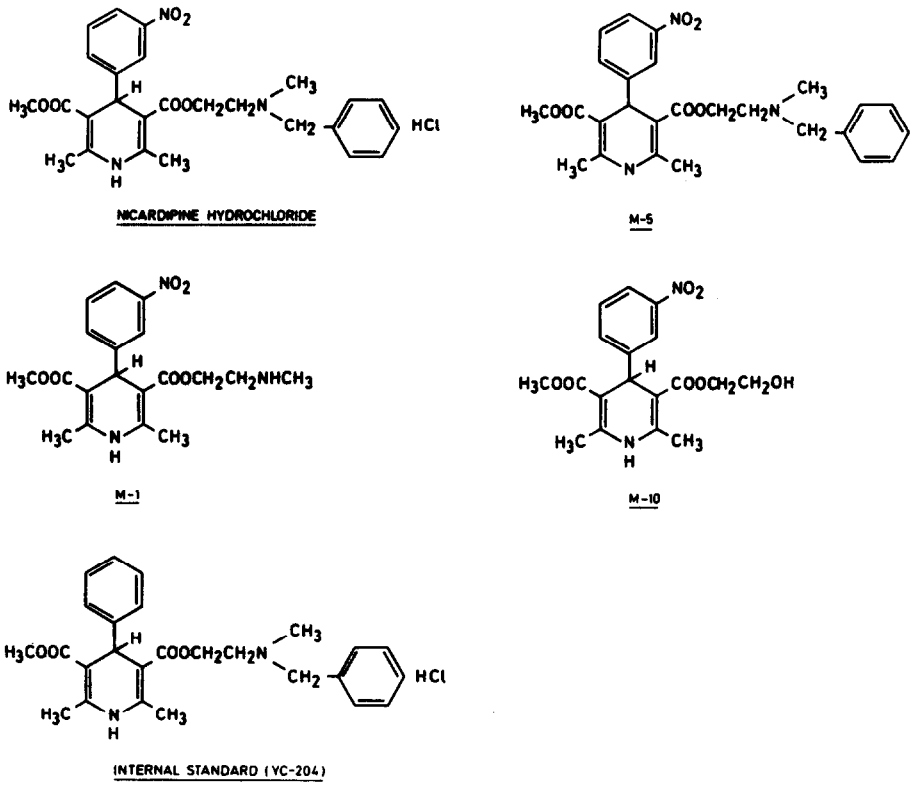

Fig. 1. Structures of nicardipine hydrochloride, its metabolites M-1, M-5 and M-10 and the internal standard (YC-204).

2. Experimental

2.1. Materials

Nicardipine hydrochloride was obtained from Sarva Syntex (Brussels, Belgium). The internal standard (I.S.) YC-204 and the metabolites M-1, M-5 and M-10 were supplied by Yamanouchi Pharmaceutical (Tokyo, Japan).

Hexane and ethyl acetate were of pesticide grade from UCB (Leuven, Belgium) and Carlo Erba (Milan, Italy), respectively. Methylene chloride, sodium hydroxide and triethylamine were of analytical-reagent grade from Merck (Darmstadt, Germany).

Stock solutions

Stock solutions I (technician I) and II (technician II) of nicardipine and the M-5 metabolite were prepared in methanol at a concentration of 1 mg/ml (equivalent to base) and were stored in brown glass tubes at −20°C. Appropriate dilutions were made. A stock solution of the I.S. (1 mg/ml) was prepared in methanol. A 1 μg/ml dilution was made.

Nicardipine and metabolite M-5 standard samples

Plasma samples of 20 ml were spiked with nicardipine and metabolite M-5 (1–50 ng/ml) using stock solution I. These plasma samples were divided into 1.5-ml portions and kept frozen at −20°C until assay.

Nicardipine and metabolite M-5 quality control samples

Plasma samples of 50 ml were spiked with nicardipine and metabolite M-5 (5, 10 and 30

ng/ml) using stock solution II. These plasma samples were divided into 1.5-ml portions and kept frozen at −20°C until assay.

At least three quality control samples (one of each concentration) were used with each set of unknown samples.

2.2. Gas chromatography

A Hewlett-Packard Model 5880 gas chromatograph, equipped with a HP on-column injector, was used. GC was performed on a 12 m × 0.32 mm I.D. Ultra 2 cross-linked 5% phenyl–methylsilicone fused-silica capillary column, with a film thickness of 0.52 μm and a phase ratio of 150 (Hewlett-Packard, Avondale, PA, USA). Samples were injected with a 10-μl Hamilton syringe and a fused-silica needle (0.18 mm O.D.). The oven temperature programme was as follows: initial temperature, 85°C for 1.00 min; programming rate, 20°C/min; final temperature, 285°C, maintained for 17 min. The detector temperature was 300°C. Helium was used as the carrier gas at a flow-rate of 6.0 ml/min and as make-up gas at a flow-rate of 20.0 ml/min. The detector was operated with hydrogen at 3.5 ml/min and with air at 75 ml/min. The peak heights were recorded on a Hewlett-Packard Model 3396A recording integrator.

2.3. Extraction procedure

The internal standard (10 ng in 10 μl of methanol) was added to 1 ml of plasma in a brown, glass-stoppered centrifuge tube. After alkalinization with 0.2 ml of 1.0 *M* Na_2CO_3 and addition of 20 μl of 0.5 *M* triethylamine in hexane–methylene chloride (1:1, v/v), the mixture was extracted twice with 3 ml of hexane–methylene chloride (1:1, v/v) by shaking horizontally for 20 min. The phases were separated by centrifugation (3015 *g*, 20 min, 4°C).

The organic phases were transferred with a Pasteur pipette into a 6-ml brown silanized glass-stoppered conical tube containing 10 μl of 2% triethylamine in ethyl acetate and evaporated to dryness in darkness under a stream of nitrogen at room temperature. The residue was reconstituted in 10 μl of ethyl acetate and 0.5 μl was used for cGC. The samples were stored at −20°C until analysis (within 48 h).

2.4. Calibration

Calibration graphs were constructed by plotting peak-height ratios of product to internal standard against the plasma standard concentration of the product. The best-fit straight line was obtained using the method of least squares and weighing factors of $1/(\text{concentration})^2$. The concentrations of the products in the unknown samples were calculated using this regression line.

2.5. Subjects

Twelve healthy volunteers received a single oral administration of 30 mg of nicardipine. Blood samples were obtained at different intervals after intake and plasma was stored at −20°C until assay.

3. Results and discussion

3.1. Chromatograms

Under the cGC conditions employed, the internal standard, metabolite M-5 and nicardipine are well separated from each other and have retention times of *ca.* 15.06, 15.30 and 21.77 min, respectively. The metabolites, M-1 and M-10, elute together at a retention time of 12.00 min. Representative chromatograms of plasma extracts are shown in Figs. 2 and 3.

3.2. Calibration

Linear relationships were found when the peak-height ratios of nicardipine or metabolite M-5 to the internal standard (y) were plotted against the plasma concentration (x). A typical regression line using $1/(\text{concentration})^2$ as weighing factor for nicardipine is $y = 0.01396 + 0.03546x$, correlation coefficient (r) = 1.0177, and for M-5 $y = 0.01529 + 0.06349x$, $r = 0.8518$.

Table 1
Accuracy and reproducibility of the cGC method for the determination of nicardipine and metabolite M-5 in standard plasma samples

Compound	Concentration (ng/ml)	R.S.D. (%)	Accuracy (%)	n
Nicardipine	1	14.0	97.9	9
	2	13.7	106.5	12
	5	12.6	101.9	12
	10	11.9	109.2	12
	20	4.6	98.2	12
	50	5.6	97.6	12
	Mean	10.4	101.8	
Metabolite M-5	1	14.5	98.0	9
	2	15.8	105.8	12
	5	12.7	101.0	12
	10	6.9	97.3	12
	20	9.8	98.5	12
	50	9.9	106.3	12
	Mean	11.6	101.1	

3.3. Accuracy and precision

The accuracy and precision of the method could be derived from back-calculated plasma standard concentrations and by replicate analysis of quality control samples. The results for the back-calculated plasma standards are summarized in Table 1. Results for the independently prepared quality control samples are summarized in Table 2.

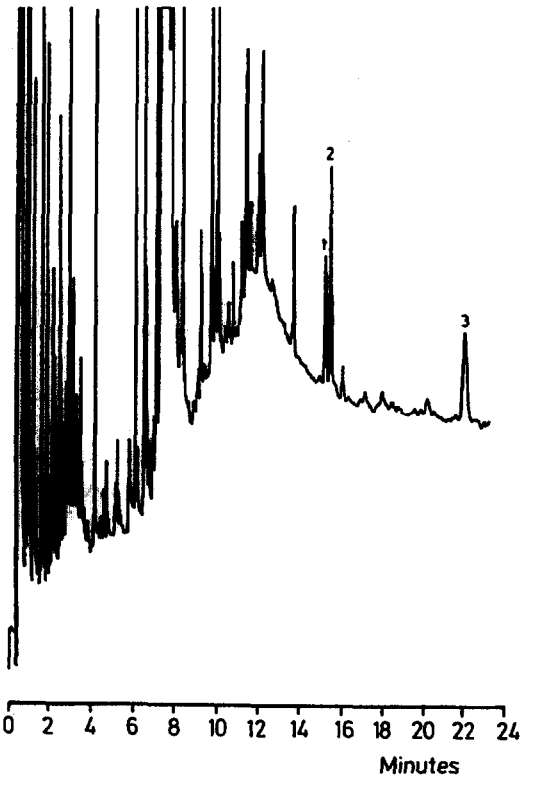

Fig. 2. Chromatogram of an extract of control human plasma spiked with 20 ng/ml of nicardipine (3), 20 ng/ml of metabolite M-5 (2) and 10 ng/ml of internal standard (1).

3.4. Detection limit

At a signal-to-noise ratio of 3, the minimum detectable concentration of both products in

Table 2
Accuracy and reproducibility of the results for quality control samples of nicardipine and metabolite M-5

Compound	Concentration added (ng/ml)	Within-day			Between-day		
		R.S.D. (%)	Accuracy (%)	n	R.S.D. (%)	Accuracy (%)	n
Nicardipine	5	10.9	104.4	5	12.4	96.1	12
	10	5.7	93.0	5	9.4	98.9	13
	30	6.7	100.5	5	8.0	104.0	13
Metabolite M-5	5				11.4	99.5	12
	10				10.0	101.1	13
	30				11.9	109.3	13

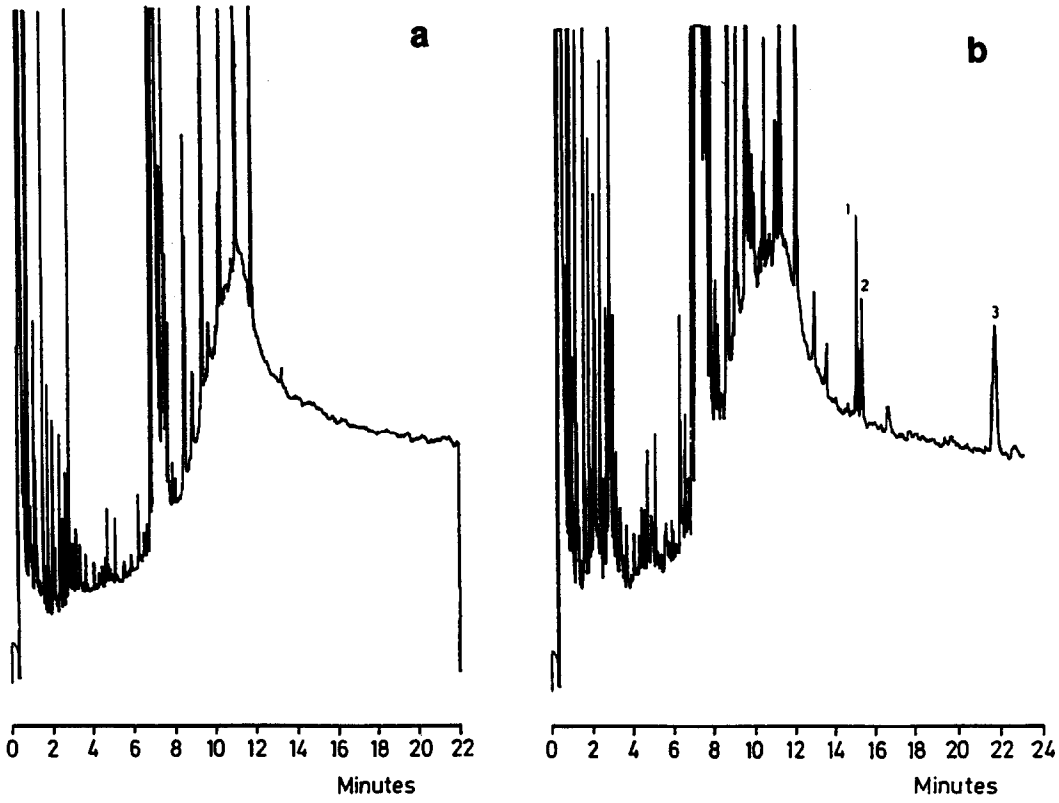

Fig. 3. Chromatograms of extracts. (a) Control human plasma from volunteer 1; (b) plasma from the same volunteer 1.5 h after a single oral dose of 30 mg of nicardipine containing 17.5 ng/ml of nicardipine (3), 7.9 ng/ml of metabolite M-5 (2) and 10 ng/ml of internal standard (1).

plasma under the conditions used was *ca.* 0.5 ng/ml.

3.5. Subjects

Mean (± S.E.M.) concentration–time profiles for nicardipine and metabolite M-5 in twelve healthy volunteers after a single oral dose of 30 mg of nicardipine are shown in Fig. 4.

4. Conclusions

Using a cross-linked 5% phenyl–methylsilicone fused-silica capillary column with on-column injection and nitrogen–phosphorus detection, a specific, sensitive and reproducible method for the determination of nicardipine and its pyridine metabolite M-5 in plasma is possible.

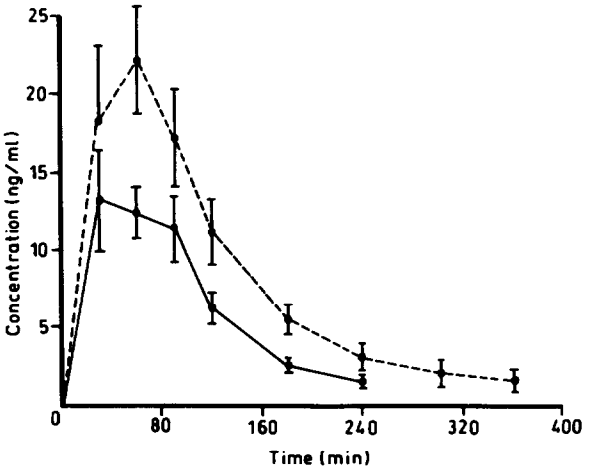

Fig. 4. Mean (± S.E.M.) concentration–time profiles for nicardipine (dashed line) and its metabolite M-5 (solid line) in twelve healthy volunteers following a single oral dose of 30 mg of nicardipine.

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