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Short Communication

Determination of isradipine and its pyridine metabolite in serum by capillary column gas chromatography with nitrogen-selective detection

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ABSTRACT

A relatively simple, sensitive and precise gas chromatographic method for the determination of isradipine, a calcium antagonist of the dihydropyridine type, and its main metabolite in serum is described. Using a one-step extraction procedure, a wide-bore column and a nitrogen–phosphorus detector, a limit of quantitation of 0.5 and 2.0 n*M* for isradipine and the metabolite was found. No interferences from several drugs were observed. The method was successfully used in a pharmacokinetic study in hypertensive women during pregnancy.

INTRODUCTION

Isradipine (PN 200-110, Lomir, DynaCirc), isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-methoxycarbonyl-2,6-dimethyl-3-pyridinecarboxylate (Fig. 1), is a new potent calcium channel blocking agent with selective action on the cardiovascular system [1]. Currently isradipine is under investigation for the treatment of hypertension, and in large studies it has given satisfactory blood pressure reduction in comparison with other antihypertensive drugs [2]. Like most other calcium channel antagonists, the bioavailability of isradipine is low, due to an extensive first-pass metabolism [3], which results in very low plasma concentrations measured in patients [3,4].

Five major metabolites have been identified in humans, all of negligible pharmacodynamic importance; the most active metabolite has an activity less than 1/1600 of that of isradipine [5]. Mea-

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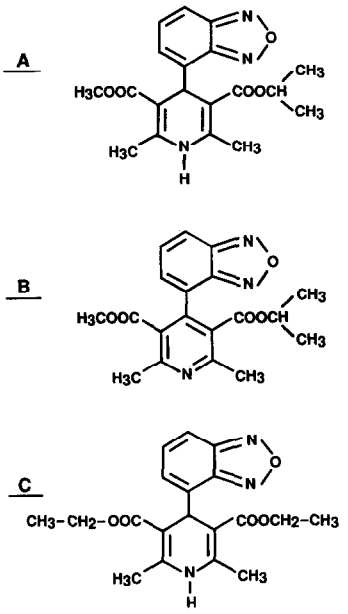

Fig. 1. Structures of isradipine (A), the pyridine metabolite 203-831 (B) and the internal standard, PY 108-068 (C).

surements of the major metabolite of isradipine could, however, be of interest in future pharmacokinetic studies, *e.g.* in multiple dosing and in patients with hepatic or renal diseases, or to detect non-compliance.

As isradipine is a relatively new drug, procedures for its assay in plasma or serum are few, although several chromatographic methods have been described for other calcium antagonists [6]. A high-performance liquid chromatographic method for isradipine has recently been published, but even though isradipine in low concentrations did cause analytical problems, it was possible to measure concentrations as low as 1.3 nmol/l (0.5 ng/ml), using a very sensitive variable-wavelength UV detector operating at maximum attenuation (0.001 a.u.f.s) [4].

As for other dihydropyridine derivatives, isradipine concentrations can be determined by gas chromatography (GC) with electron-capture detection, but for isradipine concentrations usually found in patients, a mass spectrometric (MS) detection method was necessary to obtain sufficient sensitivity (limit of detection 1.9 nmol/l (0.7 ng/ml)) [7].

The aim of the present study was to develop a relatively simple and sensitive GC method to measure isradipine and its main pyridine metabolite (Fig. 1), using a wide-bore capillary column and a nitrogen-selective detector, equipment that is available in many laboratories for drug analysis.

EXPERIMENTAL

Materials

Isradipine, its pyridine metabolite (203-831) and the internal standard (PY (108-068), Fig. 1) were donated by Sandoz (Basle, Switzerland). Stock solutions were prepared in ethanol.

All solvents and chemicals were of analytical quality. The venous blood samples were drawn into plain venoject tubes (VT-100ux, Terumo, Leuven, Belgium).

GC conditions

An HP 5890A gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a nitrogen–phosphorus detector, a capillary split–splitless injector and a 3393A integrator was used, with an HP-17, cross-linked 50% phenylsilicone capillary column (10 m × 0.53 mm I.D., 2.0 μm film thickness; Hewlett-Packard).

Procedures

A 50-μl aliquot of a 0.5 μM internal standard solution was added to 1.0 ml of serum, together with 0.5 ml of 1 *M* sodium hydroxide and extracted with 8.0 ml of *n*-hexane by horizontal shaking for 20 min. After centrifugation for 10 min at 1500 *g*, and cooling in a dry ice–acetone bath for 1 min, the organic phase was decanted into brown glass tubes and evaporated to dryness at 65°C under nitrogen. The wall of the glass tube was washed with 1 ml of toluene and evaporated to dryness. The residue was reconstituted in 20 μl of toluene, and 2 μl were analysed by GC.

The operating parameters were: injector temperature, 250°C; detector temperature, 280°C; column temperature, 90°C at injection for 2 min, then 25°C/min to 280°C for 11 min, split-off time 0–1.4 min. Gas flow-rates were: helium, 6 ml/min (carrier gas) and 25–30 ml/min (make-up gas); hydrogen, 2.0–3.5 ml/min (detector gas); air, 100–120 ml/min (detector gas). The rubidium bead power was adjusted to a signal output value of 20–30 pA.

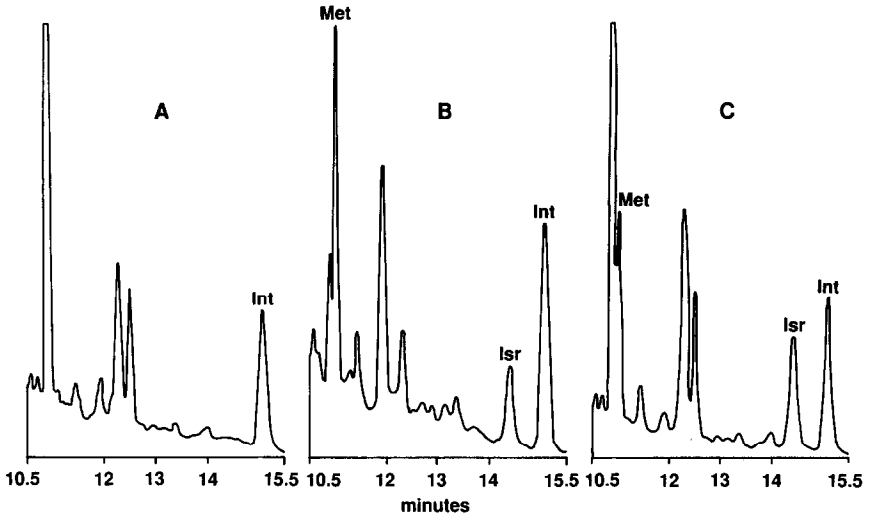

Fig. 2. Chromatograms of human serum extracts. (A) Blank serum analysed with the internal standard. (B) Serum from a patient therapeutically treated with isradipine orally. The concentrations of isradipine and its pyridine metabolite were determined as 4.6 and 11.8 nM, respectively. (C) Serum to which isradipine and its pyridine metabolite were added (10 nM/10 nM). Peaks: Int = internal standard (PY 108-068); Isr = isradipine; Met = pyridine metabolite.

Quantitation was obtained from peak-height ratio, with reference to the graphs obtained by simultaneous analysis of serum standards.

RESULTS AND DISCUSSION

The chromatograms obtained from human serum samples show a reasonable separation of isradipine, the pyridine metabolite and the internal standard from co-extracted compounds (Fig. 2). When wide-bore columns with lower polarity (HP-1 and HP-5) were used, the metabolite could not be separated from interfering peaks. With split injection and an isothermal temperature of 275°C, a better separation of the metabolite from co-extracted compounds was obtained, but the sensitivity for isradipine was less satisfactory. With splitless injection the limits of quantitation for isradipine and the metabolite were estimated to 0.5 nM (0.2 ng/ml) and 2 nM (0.8 ng/ml), respectively (coefficient of variation (C.V.) *ca.* 20%), which is better than for previously reported methods [4,7].

Calibration curves for isradipine prepared in the concentration range 1.0–75.0 nM (0.4–27.8 ng/ml) and for the metabolite in range 2–150 nM (0.8–55.4 ng/ml) were linear. The curves (n = 18) formed a mean linear regression line obtained by the least-squares method for isradipine $y = 0.014x + 0.035$ (correlation coefficient 0.99) and for the metabolite $y = 0.031x + 0.081$ (correlation coefficient 0.99), where y is the peak height of isradipine/metabolite divided by peak height of the internal standard, and x the concentration of isradipine/metabolite (nM). The intercepts

TABLE I

REPRODUCIBILITY OF REPLICATE ANALYSIS OF ISRADIPINE AND ITS PYRIDINE METABOLITE ADDED TO HUMAN SERUM

Serum concentration (n = 7, duplicate samples) (nM)		C.V. (%)
Added	Measured	
Isradipine		
2.0	1.8	14.0
8.0	7.6	5.0
15.0	14.1	4.3
30.0	30.5	3.1
Metabolite		
5.0	5.4	9.5
8.0	7.9	6.0
30.0	31.9	8.3

with the y-axis are statistically significant different from zero, $p < 0.05$, owing to interfering peaks close to those of isradipine and the metabolite.

With more polar extraction solvents, such as toluene, these and other interfering peaks were higher. Extraction recoveries with n-hexane were acceptable, *ca.* 80 and 60% for isradipine and the metabolite, respectively, when comparing peak heights obtained from extracted samples with injection of solutions of equivalent amounts. A basic pH was chosen to eliminate a lactone metabolite of isradipine [7], which otherwise would interfere with isradipine under the described GC conditions.

The venous blood samples for the serum level studies were drawn in Venoject plain tubes, since some batches of Vacutainer plain tubes showed a peak that interfered with the internal standard.

The reproducibility was estimated from spiked serum samples analysed at random on different days (Table I). The results were satisfactory and demonstrate the stability of isradipine and its metabolite in samples stored at −20°C from one day to six months.

Selectivity

Serum blanks from 25 patients with hypertension, cardiac insufficiency and/or pulmonary diseases did not show any interference from endogenous compounds, neither did those from patients undergoing treatment with the following drugs: isosorbide dinitrate, isosorbide mononitrate, nitroglycerin, diltiazem, metoprolol, potassium supplements, digoxin, furosemide, bumetanide, thiazide diuretics, spironolactone, warfarin, allopurinol, thiamazole, albuterol, ipratropium bromide, prednisolone, nitrazepam, hydralazine or diclofenac.

Codeine, salicylate, paracetamol (acetaminophen), nitrazepam and the metabolites of isradipine in solutions were added to human serum, and no interference with isradipine, its metabolite or the internal standard were observed.

Previous experience has shown that nitro-substituted dihydropyridine calcium channel blockers, such as nifedipine, are very sensitive to daylight [6]. Isradipine seems less light-sensitive, as no degradation was observed after exposure of a 1 μM ethanolic solution of isradipine to daylight for one day (data not shown).

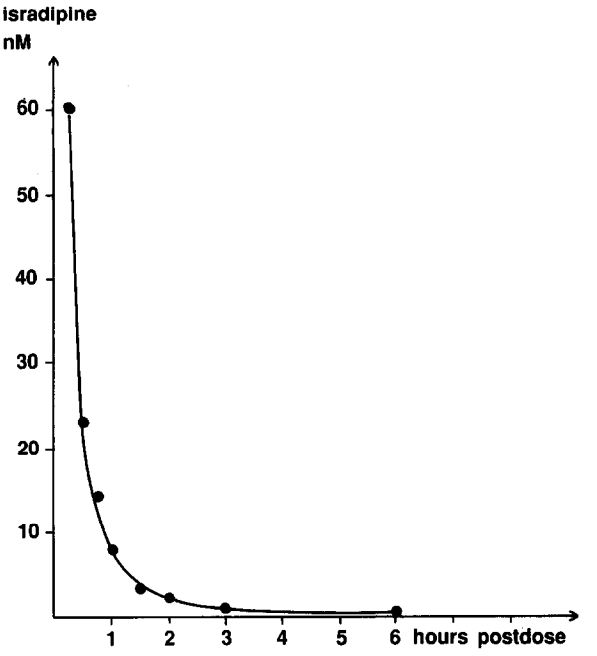

Fig. 3. Concentrations of isradipine in serum from a patient treated intravenously with 0.5 mg of the drug.

The method described has been used for the measurement of isradipine and its major metabolite in a pharmacokinetic study in hypertensive women during pregnancy. An example of the serum levels in a patient receiving 0.5 mg intravenously is presented in Fig. 3. Only trace amounts of the metabolite could be measured after intravenous administration. After oral dosing and in the steady state, metabolite levels of 5.6–38.8 nM (2.1–14.4 ng/ml) were measured.

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REFERENCES

1 R. P. Hof, R. Salzmann and H. Siegl, *Am. J. Cardiol.*, 59 (1987) 30B.
2 W. M. Kirkendall, *Am. J. Med.*, 84 (Suppl. 3B) (1988) 32.

3 A. Fitton and P. Benfield, *Drugs*, 40 (1990) 31–74.
4 J. Boutagy, F. Rumple and F. Dunagan, *J. Chromatogr.*, 487 (1989) 483.
5 R. P. Hof and U. T. Rüegg, *Am. J. Med.*, 84 (Suppl. 3B) (1988) 13.
6 M. Ahnoff and B. A. Persson, *J. Chromatogr.*, 531 (1990) 181.
7 C. Jean and R. Laplanche, *J. Chromatogr.*, 428 (1988) 61.