Journal of Chromatography, 565 (1991) 477–481
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5735

Short Communication

Determination of amidepin in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection

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(First received September 6th, 1990; revised manuscript received November 1st, 1990)

ABSTRACT

An isocratic reversed-phase high-performance liquid chromatographic method for the determination of amidepin has been developed. The method is based on the extraction of alkaline plasma with diethyl ether–dichloromethane, and the injection into the Supelcosil LC-18 column of the evaporated and reconstituted organic phase. After separation, detection is carried out by a fluorescence detector (excitation at 195 nm with no filter). The limit of detection is 10 ng/ml of plasma. The mean coefficient of variation is 12%. The plasma levels after oral administration and after intravenous administration are shown.

INTRODUCTION

Amidepin, 11-(diethylaminoacetamido)-6,11-dihydrodibenzo[*b,e*]thiepine-5,5-dioxide, is a new orginal Czechoslovak compound, proposed for use as an anti-arrhythmic agent. As amidepin is an original compound, we obtained information from the general papers [1–3]. We have developed a simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method for the measurement of amidepin in plasma with fluorimetric detection. Metoprolol was used as an internal standard.

EXPERIMENTAL

Instrumentation

Chromatography was performed on a system consisting of a Model SP 8770 pump (Spectra Physics, San Jose, CA, U.S.A.), a Model FS 970 fluorescence detector (Kratos, Ramsey, NJ, U.S.A.) and a Model 7125 injector (Rheodyne,

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Cotati, CA, U.S.A.). Chromatographic separation was carried out on a 250 mm × 4.6 mm I.D. Supelcosil LC-18 column (5 μm particle size) with a guard column (50 mm × 4.6 mm I.D.) Supelcosil LC-18 (40 μm particle size). The column and guard column were supplied from Supelco (Bellefonte, PA, U.S.A.).

Materials

Amidepin and metoprolol were supplied by the Research Institute for Pharmacy and Biochemistry (Prague, Czechoslovakia). Dichloromethane for spectroscopy was purchased from Merck (Darmstadt, Germany). Triethylamine was obtained from Fluka (Buchs, Switzerland). Methanol, diethyl ether, phosphoric acid, sodium borate, sodium hydroxide and sodium dihydrogenphosphate were supplied by Lachema (Brno, Czechoslovakia). Diethyl ether was chemically cleaned and distilled. Methanol was rectified. Water was redistilled. The extraction solvent was diethyl ether–dichloromethane (3:1, v/v).

Extraction

A 20-μl volume of the internal standard solution (4 μg/l metoprolol in redistilled water) was measured into a glass tube, to which 2 ml of plasma, 1 ml of borate buffer (0.5 *M*, pH 10) and 4.5 ml of extraction solvent were added. The tube was shaken mechanically for 15 min and centrifuged at 3000 *g* for 5 min. A 4-ml volume of the organic phase was transferred to another conical tube and evaporated to dryness. The residue was reconstituted with 100 μl of the mobile phase, and 20 μl were injected into the chromatograph.

Calibration curves

The calibration samples were prepared by using 100 μl of the amidepin solutions in 50% methanol at concentrations 0, 25, 50, 100, 250, and 500 ng/ml of plasma. The mixtures were processed as described above.

Chromatographic conditions

The mobile phase was methanol–5 m*M* sodium dihydrogenphosphate–triethylamine (30:69:1) and the pH was adjusted to 3 with phosphoric acid. Chromatographic analyses using the above solvents were carried out at a flow-rate of 1 ml/min; the temperature of the column was 40°C. The excitation wavelength of the fluorescence detector was fixed at 195 nm, and analyses were performed with no filters. The detector range was set at 0.05–0.1 μA, and the time constant was maintained at 6 s.

RESULTS AND DISCUSSION

The retention times of amidepin and metoprolol were 9.1 and 13.2 min, respectively, and the capacity factors were 2.4 and 3.9, respectively. The time of analysis was 15 min. For better accuracy the analyses were evaluated using peak heights.

TABLE I

INTRA-DAY PRECISION OF THE CALIBRATION CURVES AT FIVE DIFFERENT PLASMA CONCENTRATIONS

Amount added (ng/ml)	Amount found (mean ± S.D., n = 5) (ng/ml)	Coefficient of variation (%)
25	25 ± 5.6	22.4
50	48 ± 4.3	9.0
100	104 ± 15.6	15.0
250	253 ± 11.1	4.3
500	495 ± 34.5	7.0
Mean		11.6

The intra-day reproducibility of calibration curves was studied at five different plasma concentrations (Table I). It corresponds to the equation C (ng/ml of plasma) = $-0.13 + 160.3$ (H/His) with correlation coefficient $r = 0.9953$. The calibration curves were linear in the concentration range studied. We used one-point calibration with the equation C (ng/ml of plasma) = (160 ± 19 (H/His). The mean coefficient of variation was 12%.

Day-to-day precision of the calibration curve over three days is shown in Table II. The chromatograms of an extracted blank plasma sample, an extracted calibration plasma sample at the calibration point 250 ng amidepin per ml plasma, and a plasma sample of a healthy volunteer who received 100 mg amidepin (1 h after oral administration) are shown in Fig. 1. There is a major metabolite of amidepin in plasma appearing in Fig. 1C (retention time 7.4 min). This metabolite has not been identified so far.

The recovery test was performed at the calibration points 25, 100 and 250 ng/ml of plasma (Table III).

TABLE II

DAY-TO-DAY PRECISION OF THE CALIBRATION CURVE OVER THREE DAYS

Amount added (ng/ml)	Amount found (mean ± S.D., n = 3) (ng/ml)	Coefficient of variation (%)
25	24 ± 5.1	21.3
50	49 ± 4.5	9.2
100	101 ± 12.3	12.1
250	250 ± 15.3	6.1
500	497 ± 30.0	6.0
Mean		10.9

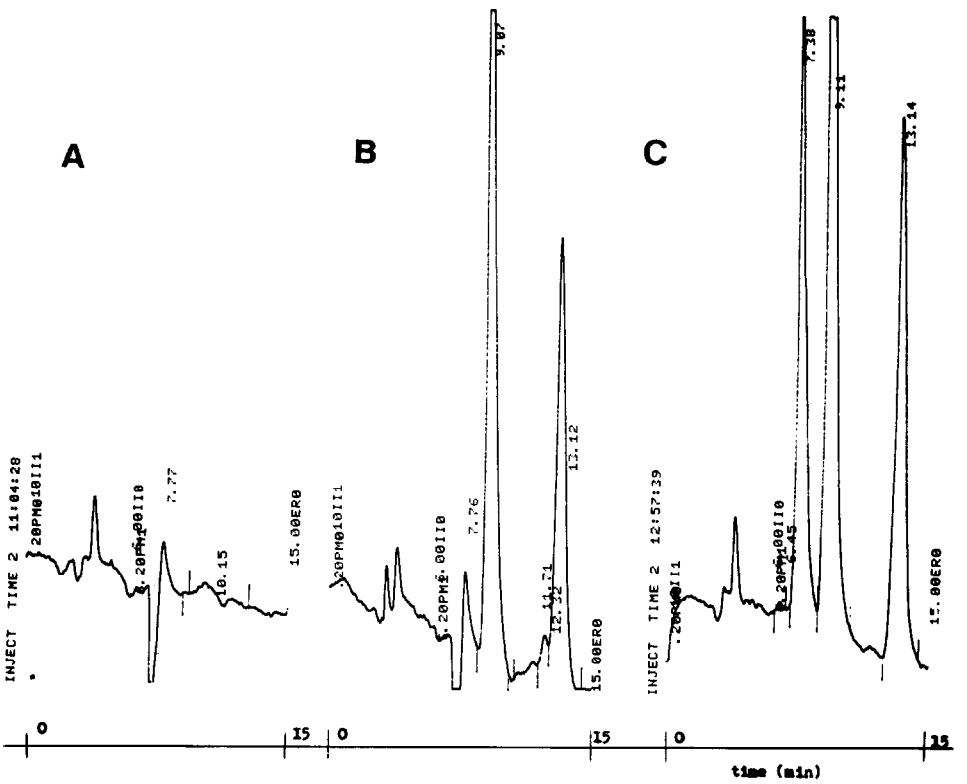

Fig. 1. Chromatograms of (A) a blank plasma sample, (B) a plasma sample spiked with amidepin (250 ng/ml) and metoprolol (40 ng/ml), and (C) a plasma sample from a healthy volunteer who received amidepin (1 h after oral administration of 100 mg). Retention times: amidepin, 9.1 min; metoprolol, 13.1 min; metabolite, 7.4 min.

TABLE III

RECOVERY OF AMIDEPIN AS A RATIO OF THE PEAK HEIGHT OF THE PLASMA SAMPLE TO THE STANDARD SOLUTION

Amount added (ng/ml)	Recovery (mean ± S.D., n = 3) (%)	Coefficient of variation (%)
25	66 ± 15.8	23.9
100	72 ± 8.1	11.3
250	69 ± 5.9	8.6
Mean	69 ± 9.9	14.6

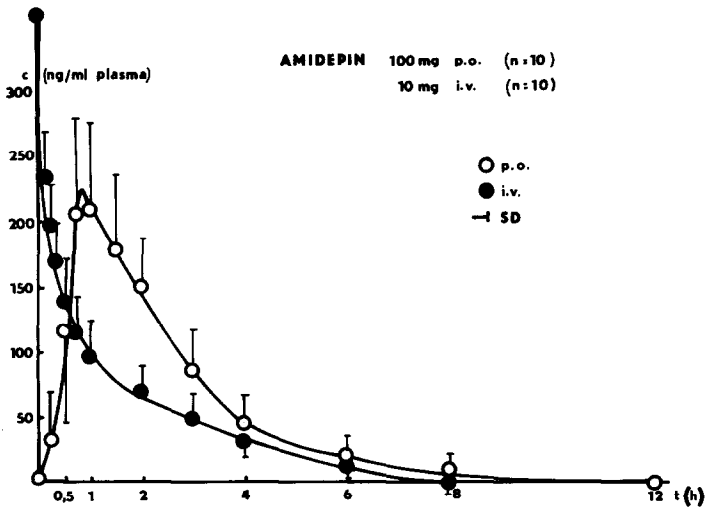

Fig. 2. Plasma concentration–time profiles of amidepin after oral administration of 100 mg and intravenous administration of 10 mg.

Amedepin is stable in frozen plasma (−18°C) for at least two months. This test was carried out at the calibration point 250 ng/ml of plasma (98 ± 8%, $n = 5$). The limit of detection was 10 ng/ml of plasma.

With regard to further phases of clinical examination, we tested for the interferences of other drugs. The test was performed by the addition of the drug (in the amount corresponding to the mean therapeutic range) to plasma, and the sample was anlysed without the internal standard. The following drugs were tested: metazosin, prazosin, labetalol, propranolol, furosemide, chlorthalidon, quinine, quinidine, naphthidrofurylic acid. None of these drugs interfered with the determination of amidepin when using metoprolol as the internal standard.

The described method was applied to samples from the first phase of clinical examination of amidepin. The plasma levels were in the range 10–250 ng/ml of plasma after a one-shot oral administration of 100 mg of amidepin (Fig. 2). The plasma levels after intravenous administration of 10 mg amidepin were in the range 10–500 ng/ml of plasma (Fig. 2).

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