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Short Communication

Determination of a new 2-amino-2-oxazoline (COR 3224) in plasma and brain tissue of the rat by high-performance liquid chromatography with electrochemical detection

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ABSTRACT

A reversed-phase (CN as stationary phase) liquid chromatographic method with electrochemical detection is described for the quantitation of COR 3224, a new 2-amino-2-oxazoline in plasma and brain tissue of the rat. Extraction was performed with dichloromethane and detection was achieved at a working electrode potential of +0.85 V *versus* an Ag/AgCl reference electrode. The recovery of the method is about 80 and 60% for plasma and brain, respectively. The limit of detection was less than 10 ng/ml for both plasma and brain, five times lower than that with ultraviolet detection.

INTRODUCTION

A new 2-amino-2-oxazoline [4,5-dihydro-5-(methyl-(4-phenyl-1-piperazenyl))-2-oxazolamine (I, Fig. 1)] has been proposed as an antidepressant agent and is currently under research [1]. The analytical method proposed for its assay in plasma [2] does not allow detection of low levels in target tissues, such as the brain.

This paper describes a new procedure for the determination of I in plasma and brain tissue of the rat.

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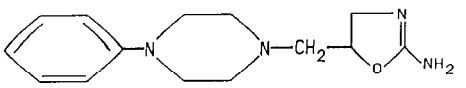

I

Fig. 1. Molecular structure of I (COR 3224): $C_{14}H_{20}N_4O$ (MW = 260.34).

EXPERIMENTAL

All chemicals used were analytical grade or HPLC grade. Plasma extraction of I (either drug sample or spiked plasma) was performed by dichloromethane after alkalinization by Tris buffer (0.2 *M*, pH 10). The internal standard was the *o*-O-methyl-substituted derivative of I. After evaporation of the organic phase the residue was dissolved in 200 μl of methanol, and 5–10 μl were injected into the chromatographic system. Extraction of I from brain tissue was performed after homogenization of whole brain (1.2–1.5 g) for 15 s in an Ultra-turrax homogenizer (Jahne & Kunkel, Staufen, F.R.G.) in 10 ml of dichloromethane. The organic phase was evaporated under nitrogen at room temperature, and the residue was dissolved with 1 ml of Tris buffer and then extracted as described above for plasma samples.

The chromatographic system consisted of a Beckman 116 constant-flow pump (Beckman, San Ramon, CA, U.S.A.), a reversed-phase column (75 mm × 4.6 mm I.D., Ultrasphere CN 3 μm, Beckman) and a Metrohm 641-VA electrochemical detector (Metrohm, Herisau, Switzerland). Injection was performed with a high-pressure sampling valve (Rheodyne 7125 with a 20-μl loop), and peaks were recorded on a chart recorder (Kipp & Zonen, Delft, The Netherlands).

The mobile phase was methanol–0.1 *M* ammonium acetate (10:90, v/v) containing triethylamine (0.4%). The pH was adjusted to 5.5 with concentrated phosphoric acid. The flow-rate was 1.4 ml/min and the potential of the working electrode (glassy carbon) was set +0.85 V against the Ag/AgCl reference electrode.

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms of spiked plasma and brain samples. The two peaks were well separated, with retention times of 2.5 min for I and 4 min for the internal standard. The standard curves for I were linear in the tested concentration range (125–2000 ng/ml for plasma and 125–1000 ng/g for brain). The equations of the calibration curves were $y = 0.0102 + 0.0024x$ ($r = 0.999$) and $y = -0.0083 + 0.003x$ ($r = 0.999$) for plasma and brain tissue, respectively.

The recovery was evaluated by spiking drug-free plasma or brain samples with different amounts (125–1000 ng/ml) of I, and calculated by comparing the peak-

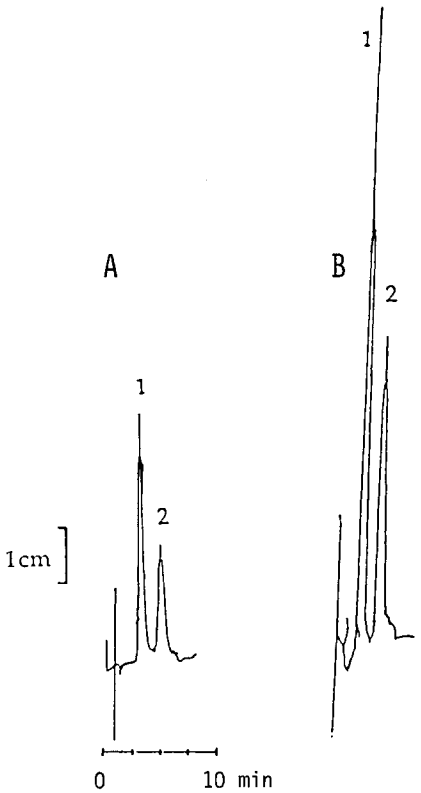

Fig. 2. Representative chromatograms of (A) extracted spiked plasma (I = 1000 ng/ml) and (B) extracted spiked rat brain (I = 1000 ng/ml). Peaks: 1 = I; 2 = internal standard.

TABLE I

RECOVERY OF THE EXTRACTION AND WITHIN-DAY PRECISION OF THE METHOD FOR I FROM SPIKED PLASMA AND BRAIN SAMPLES

Sample	*n*	Concentration added (ng/ml)	Recovery (mean ± S.E.M.) (%)	C.V. (mean ± S.E.M.) (%)
Plasma	5	125	82.1 ± 4.0	3
	5	500	80.8 ± 3.2	2.6
	5	1000	85.3 ± 2.3	2
Brain	5	125	62.6 ± 4.5	5.1
	5	500	60.3 ± 4.5	4.0
	5	1000	60.9 ± 3.8	3.1
Mean ± S.E.M.				3.46 ± 1.04

height ratio of the extract with the peak-height ratio of a standard solution injected directly. The values were *ca.* 80% in plasma and 60% in brain, whatever the concentration. It was found to be reproducible, with a variability of less than 5% (Table I).

The precision, evaluated for three different drug concentrations, is satisfactory: the within-day precision of the method showed a coefficient of variation (C.V.) of less than 6% for the lowest concentrations for spiked plasma and brain samples (Table I).

Stability studies of I in acid solution at 20°C and 4°C for 6 h and at −20°C for 6 and 16 months in rat plasma, showed that I was stable for 6 h at 20°C (100% recovery), for 1 week at 4°C (100%) in acid solution, for 6 months at −20°C (90%) and for 15 months at −20°C (94.7%) in rat plasma.

The limit of detection of the method (a signal-to-noise of 4:1) was less than 10 ng/ml I for both plasma and brain, which is five times lower than that obtained with UV detection [2].

Thus, the method described here can be used satisfactorily in pharmacokinetic studies of I in plasma and tissue samples.

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