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Note

Determination of citalopram in plasma and brain tissue of the rat by high-performance liquid chromatography with ultraviolet detection

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Citalopram (Lu 10-171, Fig. 1) is a specific potent serotonin re-uptake inhibitor [1,2], which is under clinical trials as an antidepressant [3]. Several methods, including thin-layer chromatography [4], fluorescence scanning [5] and high-performance liquid chromatography (HPLC) [6–8], for the detection of citalopram in plasma and urine samples, as well as citalopram metabolites in plasma [8], have been published. No methods are yet available for the quantitative determination of citalopram in target tissues, such as the brain. Such concentrations are, however, of utmost interest when studying the effects as well as the pharmacokinetics of an antidepressant. The study described in this paper was undertaken to develop a common procedure for the determination of citalopram in plasma and brain tissue of the rat.

EXPERIMENTAL

Standards and reagents

Citalopram hydrobromide (Fig. 1) and desipramine hydrochloride (internal standard) were supplied by Lundbeck, Copenhagen, Denmark, and Ciba-Geigy, Basel, Switzerland, respectively. All chemicals were analytical grade and HPLC

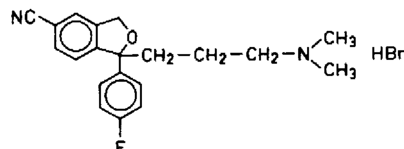

Fig. 1. Chemical structure of citalopram.

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grade. Methanol was used as solvent for stock solutions of citalopram and desipramine. The concentration of stock solutions was 1 mg/ml, and they were stored at 4°C. Aqueous solutions were prepared using glass-distilled water.

HPLC instrumentation and conditions

The HPLC system consisted of a Gynkotek 600/200 constant-flow pump (Germering, F.R.G.), a reversed-phase column (100×4 mm I.D., Spherisorb ODS C_{18} 5 μm; Knauer, Bad Homburg, F.R.G.) and a Spectroflow 773 spectrophotometer with a deuterium lamp (Kratos, Karlsruhe, F.R.G.). Automatic injection was performed by WISP 710 B autosampler (Waters, Eschborn, F.R.G.), and peaks were evaluated by an SP 4100 computing integrator (Spectra Physics, Darmstadt, F.R.G.) [9].

The mobile phase was acetonitrile–0.025 *M* potassium dihydrogenphosphate (50:50, v/v). The flow-rate was 1.5 ml/min and the detection wavelength 240 nm. Chromatography was carried out at room temperature (21–22°C).

Sample preparation

Male Wistar rats (WISW, SPF Cpb; Winckelmann, Borchen-Kirchborchen, F.R.G.) of 120–150 g body weight were given a single intravenous (i.v.) dose of citalopram (10 mg/kg). Drug-treated or control animals were sacrificed by decapitation, and blood was obtained from the cervical trunk and collected in heparinized tubes. After centrifugation the plasma was pipetted off and kept at -25°C. The forebrains were quickly removed, divided sagittally in the midline, rinsed in ice-cold 0.9% saline solution, blotted on filter paper, weighed, frozen in liquid nitrogen and finally stored at -25°C as described earlier [9].

Extraction from plasma

To 1 ml of plasma (either drug sample or spiked plasma) were added 100 μl of desipramine solution (1–2 μg/ml) as internal standard, 1 ml of 1 *M* sodium hydroxide, 250 mg of sodium chloride and 6 ml of dichloromethane. The tubes were shaken for 15 min, and after centrifugation at 1500 *g* for 15 min the aqueous phase was discarded. Then 5 ml of the remaining organic phase were transferred to a conical glass tube and evaporated under a gentle stream of nitrogen at 37°C in a water-bath. The residue was redissolved in 200 μl of mobile phase. Finally, 100–150 μl of the clear solution were injected into the column for HPLC analysis.

Extraction from brain tissue

The brain samples (hemiforebrain, 0.4–0.6 g wet weight) were gently homogenized with an Ultra-Turrax homogenizer (Jahnke & Kunkel, Staufen, F.R.G.) in 2 volumes (w/v) of 0.9% saline solution. Then 1 ml of the homogenate was transferred to a glass tube and extracted as described above for plasma samples.

Recovery studies

The accuracy of the method was evaluated by spiking two to five drug-free plasma or brain samples with different amounts (100–2000 ng/ml) of citalopram, followed by the extraction procedure. The recovery was calculated by comparing

peak-height ratios of these extracts with peak-height ratios of a standard solution injected directly.

The within-day precision of the method was calculated by the coefficient of variation (C.V.) obtained from six experiments with four to ten aliquots of plasma or brain samples.

RESULTS AND DISCUSSION

The method described provides a simple and easy-to-handle method for the quantitative determination of citalopram, not only in plasma but also in target tissue such as the brain. Blank plasma and brain samples obtained from several rats were tested for absence of interfering endogenous plasma and brain components. Fig. 2a and Fig. 3a show typical chromatograms of blank plasma and brain samples, and Fig. 2b and Fig. 3b are from spiked samples, respectively. Two peaks are separated nearly at baseline with a retention time of 9.3 min for citalopram and 12.2 min for desipramine (internal standard). Figs. 2c and 3c demonstrate that the method allows the separation and identification of citalopram and desipramine in rat plasma and brain 15 min after i.v. injection of citalopram (10 mg/kg). As with other lipophilic compounds, such as propranolol [10] and imipra-

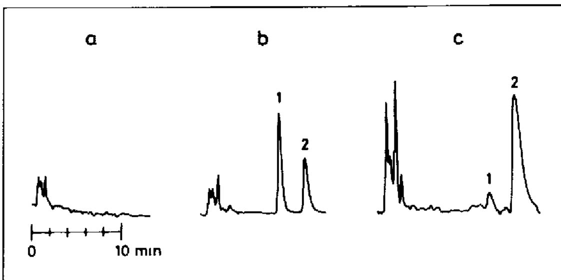

Fig. 2. Representative chromatograms of rat plasma: (a) blank sample; (b) spiked sample (citalopram 250 ng/ml); (c) plasma collected 15 min after i.v. injection of 10 mg/kg citalopram, peak represents 117 ng/ml citalopram. Peaks: 1 = citalopram; 2 = desipramine (internal standard).

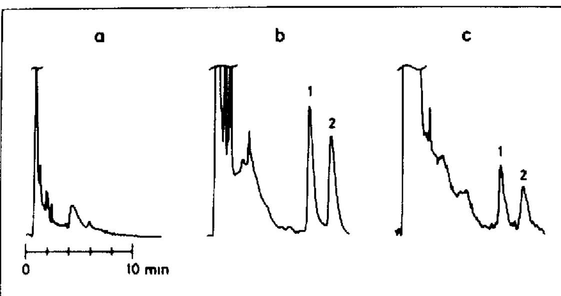

Fig. 3. Representative chromatograms of rat forebrain samples: (a) blank sample; (b) spiked sample (citalopram 750 ng/ml); (c) brain sample 15 min after i.v. injection of 10 mg/kg citalopram, peak represents 799.8 ng/g citalopram. Peaks: 1 = citalopram; 2 = desipramine (internal standard).

mine [11], higher concentrations of citalopram are found in brain than in plasma (Figs. 2c and 3c).

The standard curves for citalopram showed linearity in the tested concentration range 100–2000 ng/ml (Fig. 4). The equations of the calibration curves for plasma and brain tissue were: $y=0.0047x-0.17$, $r=0.9982$ and $y=0.0020x-0.046$, $r=0.9968$, respectively. The different slopes for plasma and brain tissue are caused by the different recoveries of the lipophilic internal standard desipramine.

The recovery and precision of the method are presented in Tables I and II. The substances were added to drug-free plasma or brain samples and analysed by the extraction procedure. The recovery was tested by comparison of the peak heights of extracted samples with peak heights obtained by spiking known amounts of citalopram into the column. The precision of the method was evaluated with different drug concentration. For each concentration a set of five specimens was

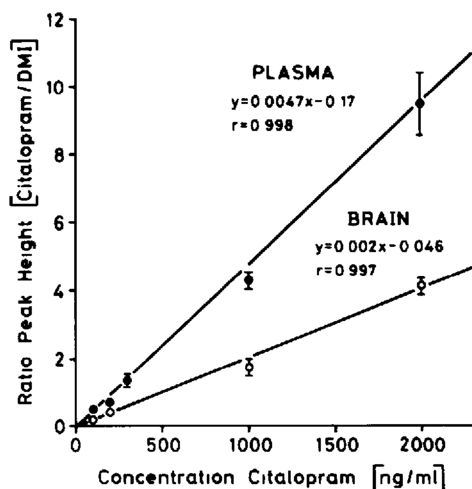

Fig. 4. Standard curves of citalopram for plasma and brain tissue. Desipramine (DMI) was added as internal standard. Mean values ± S.E.M. of three to five independent samples. The equations of the calibration curves are shown.

TABLE I

RECOVERY OF THE EXTRACTION METHOD FOR CITALOPRAM FROM SPIKED PLASMA AND BRAIN SAMPLES

Sample	Number of samples	Citalopram added (ng/ml)	Recovery (mean ± S.E.M.) (%)
Plasma	3	100	75.5 ± 4.1
	2	1000	75.3 ± 0.4
	5	2000	71.0 ± 3.0
Brain	4	250	81.4 ± 1.0
	4	500	74.0 ± 2.6
	5	1000	77.9 ± 3.2

TABLE II

WITHIN-DAY PRECISION OF THE METHOD

Sample	Number of samples	Citalopram added (ng/ml)	C.V. (%)
Plasma	5	300	5.0
	4	500	1.5
	10	1000	3.7
Brain	4	250	5.5
	5	500	6.8
	5	1000	7.0
Mean ± S.E.M.			4.9 ± 0.8

analysed within one day as described. The coefficients of variation (relative S.D.) calculated for spiked plasma and brain samples are listed in Table II. The within-run accuracy of the assay determined in six experiments with with four to ten aliquots of plasma or brain samples was 4.9 ± 0.8% (Table II). The limit of detection of the method was less than 25 ng/ml citalopram for both plasma and brain; for this minimum concentration a signal-to-noise ratio of 4:1 was observed. Although the "basic–acidic" extraction method was not used in this study, the recovery of citalopram was almost the same as reported previously [6,7]. The use of dichloromethane as extraction solvent resulted in a sufficiently high recovery of citalopram without interference from the coextracted plasma and brain components. Less polar solvents, such as heptane and hexane, extracted citalopram with low efficiency. The extraction with dichloromethane is simple and there is no difference in the procedure whether plasma or brain tissue is analysed, as is often necessary with other lipophilic compounds [9,10]. Thus, the HPLC method described can easily be used in pharmacokinetic studies investigating citalopram in plasma as well as tissue samples.

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