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Short Communication

Measurement of trazodone in plasma and brain of rat by capillary gas chromatography with a nitrogen-selective detector

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ABSTRACT

A specific and highly sensitive method for the measurement of trazodone in plasma and brain of rat is presented. The compound and the internal standard were extracted from alkalinized samples with hexane and analysed by capillary gas chromatography with nitrogen-selective detection. The method was demonstrated to be accurate and precise. The limits of determination were 2 ng/ml for plasma and 24 ng/g for brain, which makes this procedure suitable for pharmacokinetic analysis.

INTRODUCTION

Trazodone, 2-[3-(4-*m*-chlorophenyl-1-piperazinyl)propyl]-1,2,4-triazolo[4,3-α]pyridin-3-(2*H*)-one (Fig. 1), a triazolopyridine derivative, is an atypical antidepressant drug which is purported to have a greater margin of safety than tricyclic antidepressant drugs. Previously reported methods used to monitor the levels of trazodone in plasma and tissues are high-performance liquid chromatography with UV [1–4] or electrochemical detection [5,6] and gas chromatography (GC) with flame-ionization detection [7], nitrogen-selective detection [8–10] or mass fragmentographic detection [11,12]. Most of these methods were sensitive and specific enough for toxicological studies and therapeutic drug monitoring (0.1–0.25 μg/ml), but appear to be inadequate for pharmacokinetic studies. Some [5,12] are most sensitive but do not allow to trazodone to be determined at levels lower than 0.01 μg/ml.

We present here a GC method with nitrogen–phosphorus detection which allowed plasma levels of trazodone as low as 2 ng/ml to be determined. This was made possible by combining a capillary column and a jet designed to reduce the

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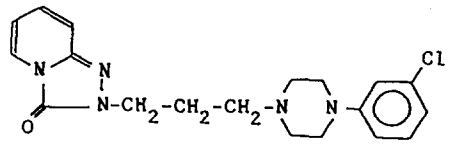
Trazodone



I.S.

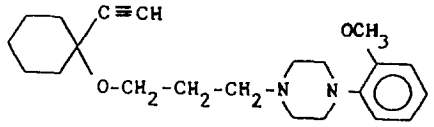


Fig. 1. Structures of trazodone and I.S.

pressure at the detector inlet, as a high flow-rate of helium (3–4 ml/min) was found to be necessary for such trazodone determinations when performed with a capillary column.

EXPERIMENTAL

Reagents

Trazodone was obtained from Sigma (La Verpillière, France) and 1-[(1-ethynylcyclohexyl)-oxy]-3-[4-(2-methoxy)phenyl-1-piperazinyl]-propane (Fig. 1) was used as internal standard (Parcor, French Patent No. 2328469).

All reagents and solvents were of analytical-reagent grade purity. Hexane was obtained from Merck (Darmstadt, Germany) and methanol from Carlo Erba (Milan, Italy).

Standard solutions of trazodone and the I.S. were prepared in methanol (1 mg/ml). Working solutions (0.1–100 μg/ml) were freshly prepared before analysis by dilution in methanol.

Gas chromatography

The determinations were carried out on a Hewlett-Packard Model 5890 gas chromatograph equipped with a nitrogen–phosphorus detector. The capillary column (12 m × 0.20 mm I.D.) was HP-1 cross-linked methylsilicone gum (Hewlett-Packard, Les Ulis, France). Helium was used as the carrier gas, at a pressure of *ca.* 2 bar. With regard to the high flow-rate of helium which was necessary for GC of trazodone, the jet had a 0.046-cm tip. Hydrogen, air and nitrogen flow-rates were 4, 100 and 30 ml/min, respectively. The column was maintained at 60°C for 1 min, then programmed at 45°C/min to 240°C, at 10°C/min to 260°C and finally at 3°C/min to 280°C. The injector and detector temperatures were 250 and 280°C, respectively.

Animals and sample collection

Experiments were carried out on male Wistar rats (280–320 g). The animals used in acute experiments were deprived of food for 18 h before each experiment, but they had free access to water. The rats were treated with trazodone hydrochloride (15 mg/kg intravenously) and decapitated at various times. Blood was collected in a citrated tube (100 μl of a 190 mg/ml solution) and centrifuged for 10 min at 1600 *g*. Plasma and brain specimens were kept frozen at −20°C until analysed.

Extraction from plasma

Plasma samples (1 ml), spiked with 100 ng of I.S. (10 μl from a 10 μg/ml working solution), were placed in a 15-ml glass centrifuge tube with 100 μl of 2 *M* sodium hydroxide solution and extracted twice with 2 ml of isoamyl alcohol–hexane (1.5:98.5, v/v) by mechanical shaking for 10 min. After centrifugation at 1600 *g* for 10 min, the organic layer was evaporated to dryness, using a Speed Vac concentrator (Savant SC 100, Bioblock, France). The residue was dissolved in 25 μl of methanol and 2 μl were injected for GC analysis.

Extraction from brain

Brains were homogenized in 0.1 *M* acetic acid (5 ml/g), then 1 ml of this homogenate was transferred into a 15-ml glass centrifuge tube, spiked similarly with 100 ng of I.S. and made alkaline with 500 μl of 2 *M* sodium hydroxide solution, and finally extracted with 2.5 ml of hexane. In order to eliminate endogenous lipidic compounds, 1 ml of 0.1 *M* hydrochloric acid was added to the organic layer and shaken mechanically for 10 min. After centrifugation for 10 min at

1600 *g*, the aqueous layer was separated, made alkaline with 500 μl of 2 *M* sodium hydroxide solution and re-extracted with 2 ml of hexane by mechanical shaking for 10 min. After a final centrifugation for 10 min at 1600 *g*, the organic layer was evaporated to dryness using a Speed Vac concentrator. The residue was dissolved in 25 μl of methanol and 2 μl were injected for GC analysis.

Specimens containing trazodone at levels greater than 12 μg/g were re-analysed after dilution with a blank brain homogenate.

RESULTS AND DISCUSSION

Linearity

Over the range of concentrations studied, *i.e.*, from 2 ng/ml to 10 μg/ml for plasma and from 24 ng/g to 12 μg/g for brain, the linearity was fairly satisfactory despite this broad range. Linear regression equations of the mean plots ($n = 7$) were $y = 0.0166x - 0.33$ for brain and $y = 0.062x - 0.042$ for plasma, with correlation coefficients >0.990 and relative standard deviations (R.S.D.) of the slope of 9.1 and 12.5%, respectively. However, the lower values were not precise and accurate enough when calculated with these calibration graphs. Therefore, two additional calibration graphs were plotted, from 2 to 20 ng/ml for plasma and from 24 to 240 ng/g for brain. The linear regression equations of these plots ($n = 7$) were $y = 0.0094x - 0.0014$ for plasma and $y = 0.0035x - 0.0003$ for brain, with R.S.D.s of the slopes of 18.3 and 16.0%, respectively. In the above equations, y is the peak-area ratio of trazodone to the I.S. and x is the concentration of trazodone.

Precision and accuracy

The within-batch (intra-assay) validation of the assay was carried out by analysing replicate samples ($n = 7$) prepared in control matrix with different concentrations of trazodone in the calibration range. Between-batch validation was carried out by analysing plasma and brain samples at different concentrations on seven different days over a period of two months. The R.S.D. was used as a measure of the precision and the relative difference between found and added amounts as a measure of the accuracy. The results are given in Table I.

Extraction recoveries

The extraction recoveries were 89.9 ± 8.2% for a plasma sample spiked at 100 ng/ml ($n = 6$) and 58.9 ± 6.1% for a brain sample spiked at 600 ng/g ($n = 6$). The low yield of brain tissue could be related to the procedure used to extract trazodone. The extraction procedure for brain samples included an extraction of trazodone in two steps, with consequent losses. However, this procedure was necessary to produce good chromatograms.

Limits of determination

The limits of determination under the experimental conditions described were 2 ng/ml for

TABLE I

PRECISION AND ACCURACY OF THE MEASUREMENT OF TRAZODONE IN RAT PLASMA AND BRAIN SAMPLES

Amount added	Amount recovered (mean ± S.D.)	R.S.D. (%)	Relative mean error (%)
Intra-day			
Plasma (ng/ml)			
2	1.9 ± 0.2	12.4	−10.4
100	105.6 ± 3.1	2.9	+5.6
10 000	10 450 ± 291	2.8	+4.5
Brain (ng/g)			
24	23.3 ± 3.4	14.7	−11.2
600	631 ± 52	8.2	+6.4
12 000	13 468 ± 1596	11.8	+12.2
Inter-day			
Plasma (ng/ml)			
2	2.1 ± 0.2	8.6	+3.0
100	100.3 ± 5.6	5.5	+0.1
10 000	10 205 ± 182	1.8	+2.0
Brain (ng/g)			
24	23.3 ± 0.9	3.9	−0.1
600	599 ± 54	1.5	−0.1
12 000	12 261 ± 606	4.9	+2.2

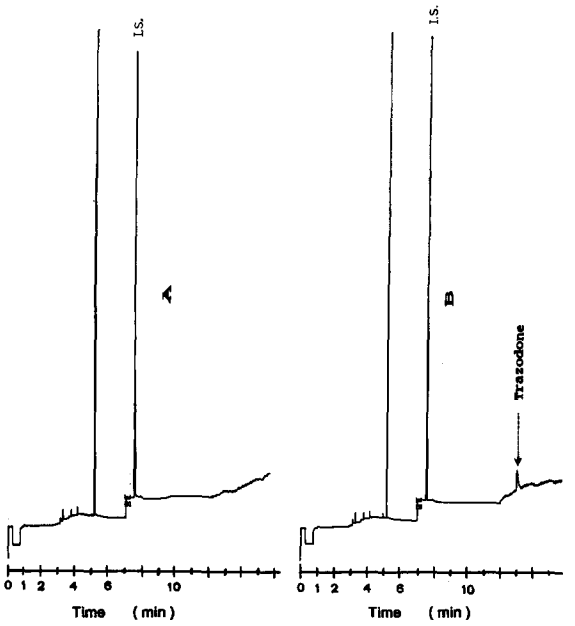


Fig. 2. Chromatograms of plasma samples (A) not supplemented and (B) supplemented with 2 ng/ml trazodone.

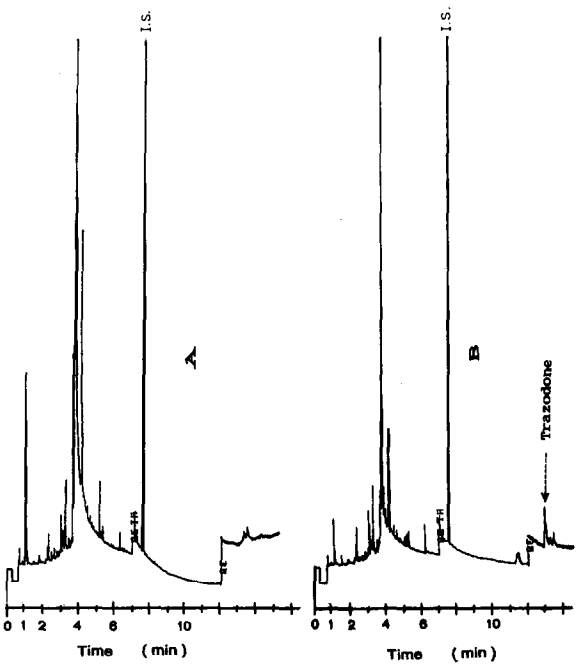


Fig. 3. Chromatograms of brain samples (A) not supplemented and (B) supplemented with 24 ng/g trazodone.

plasma and 24 ng/g for brain. This difference may be due to the sample itself, the solvents and the two-step procedure used for brain extraction. Owing to the values of the R.S.D. (<20%) and the relative error (<15%), the values indicated above were obviously validated as limits of quantitation. Chromatograms of plasma and brain samples supplemented or not with trazodone at the limits of determination are shown in Figs. 2 and 3.

Analyte stability

Stability in plasma was checked by analysing duplicate samples at 2 ng/ml, 100 ng/ml and 10 μg/ml after keeping them frozen at −20°C for ten days, one month and two months. The mean standard errors were for 2 ng/ml, 100 ng/ml and 10 μg/ml, respectively, −2.5, +5.2, +4.2% (ten days), −4.0, −1.8, −3.8% (one month) and +13.0, +4.3, +5.1% (two months).

Animal study

Fig. 4 shows the curves of mean plasma and

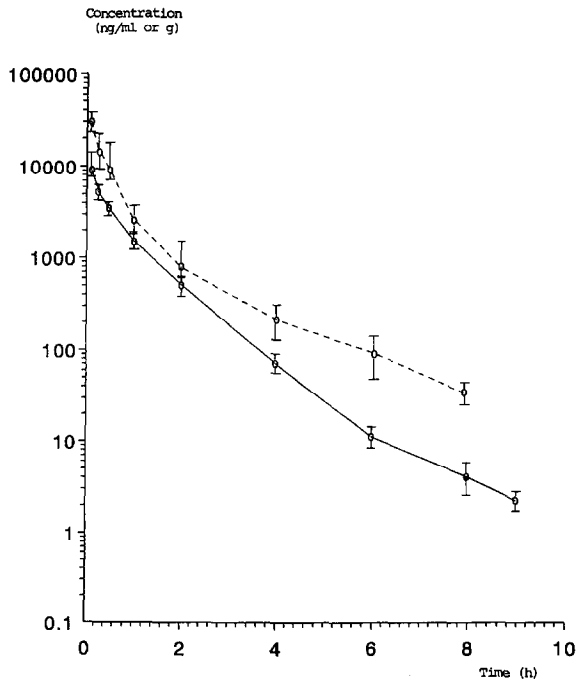


Fig. 4. Mean plasma (solid line) and brain (dashed line) concentrations (with standard deviations) of trazodone after intravenous administration of trazodone (15 mg/kg). Each point is the mean for six rats.

brain concentrations of trazodone over 8 h. Each point is the mean for six rats. The plasma and brain half-lives were similar, *ca.* 1.5 h. Previously reported methods gave a shorter half-life in rats, *i.e.*, 50 min [4]. This could be related to the sensitivity of our method, which allowed the kinetics to be followed in the rat over an 8-h period. Accordingly, the method presented here appears to be accurate enough to follow the pharmacokinetics of trazodone.

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