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GAS CHROMATOGRAPHIC–MASS FRAGMENTOGRAPHIC DETERMINATION OF TRAZODONE IN RAT PLASMA

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SUMMARY

A sensitive and specific combined gas chromatographic–mass fragmentographic method for the determination of trazodone in rat plasma is described.

After extraction with diethyl ether and washing of the extracts in order to prevent interference from endogenous materials, trazodone and the internal standard benperidol were separated on an OV-1 column. The minimum amount of trazodone detected was 20 ng by gas chromatography with the use of a flame ionization detector and 200 pg when using the mass fragmentographic technique. Plasma levels in rats treated with a single intravenous dose (10 mg/kg) of trazodone are also reported.

INTRODUCTION

Trazodone, 2-{3-[4-(*m*-chlorophenyl)-1-piperazinyl]propyl}-*s*-triazolo[4,3*a*]pyridin-3-(2H)-one, is a new psychotropic drug[1–4] with antidepressant, sedative and analgesic properties[5–7]. The levels of trazodone in plasma and tissues have been assayed by spectrofluorimetry[8], gas chromatography (GC)[9] and by using the ^{14}C-labelled compound[10], although these methods lack sensitivity and/or specificity, particularly when applied to biological specimens. The method described here does not have these disadvantages because it combines the high resolving power of the gas chromatograph with the high sensitivity and specificity of the identification provided by the mass spectrometer.

As an example, this method has been applied to detection of the plasma level of trazodone after its administration to rats in a single dose.

EXPERIMENTAL

Standards and reagents

Trazodone was supplied by Angelini (Rome, Italy) and benperidol (used as the internal standard) by Janssen (Beerse, Belgium). Trazodone and benperidol were used as their hydrochloride salts, with all concentrations expressed in terms of the free base.

The following reagents were used: sodium hydroxide, hydrochloric acid, methanol, diethyl ether and chloroform (Carlo Erba, Milan, Italy).

Gas chromatography

GC was carried out on a Carlo Erba Fractovap Model G1 gas chromatograph, equipped with a flame ionization detector (FID). The column consisted of a glass tube, 80 cm long and 4 mm I.D., packed with 1 % OV-1 on Chromosorb Q, 100–120 mesh, conditioned for 1 h at 280° (30 ml/min nitrogen flow), 4 h at 310° (no nitrogen flow) and 24 h at 240° (35 ml/min nitrogen flow).

The operating conditions were: column temperature, 240°; injector port temperature, 270°; and carrier gas (nitrogen) flow-rate, 35 ml/min. The air and hydrogen flow-rates were adjusted so as to provide maximum response.

Gas chromatography–mass fragmentography (GC–MF)

An LKB Model 9000 mass spectrometer equipped with a gas chromatograph was used. The chromatographic conditions were as described above and the carrier gas (helium) flow-rate was 35 ml/min. The mass spectrometer was set to meet the following conditions: energy of the ionization beam, 70 eV; ion source temperature, 290°; accelerating voltage, 3.5 kV; and trap current, 60 μA.

MF measurements were performed by focusing the instrument on the ions at *m/e* 205 for trazodone and at *m/e* 187 for benperidol, which are characteristic and present within a mass range of 10 % of each other, in accordance with the technical requirements of the instrument.

Procedure for extraction from water and plasma

The procedure reported below was utilized to measure trazodone in the range between nanograms and micrograms.

To 1 ml of water or 1 ml of plasma, 0.5 ml of 1 *N* sodium hydroxide solution and 7 ml of diethyl ether were added. The tubes were shaken gently for 30 min and then centrifuged at 4° for 10 min. A 6.5-ml volume of the organic layer was transferred into a second test-tube and evaporated to dryness under a gentle stream of nitrogen.

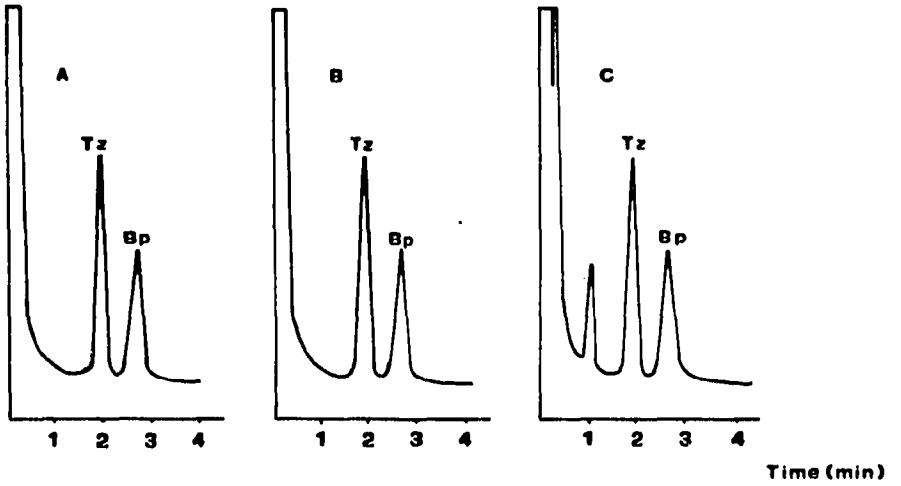

Fig. 1. Gas chromatograms obtained by using the flame ionization detector. (A) Standard trazodone (Tz) and benperidol (Bp) (internal standard). (B) Water extract. (C) Plasma extract. All other peaks present in the chromatograms are due to endogenous substances.

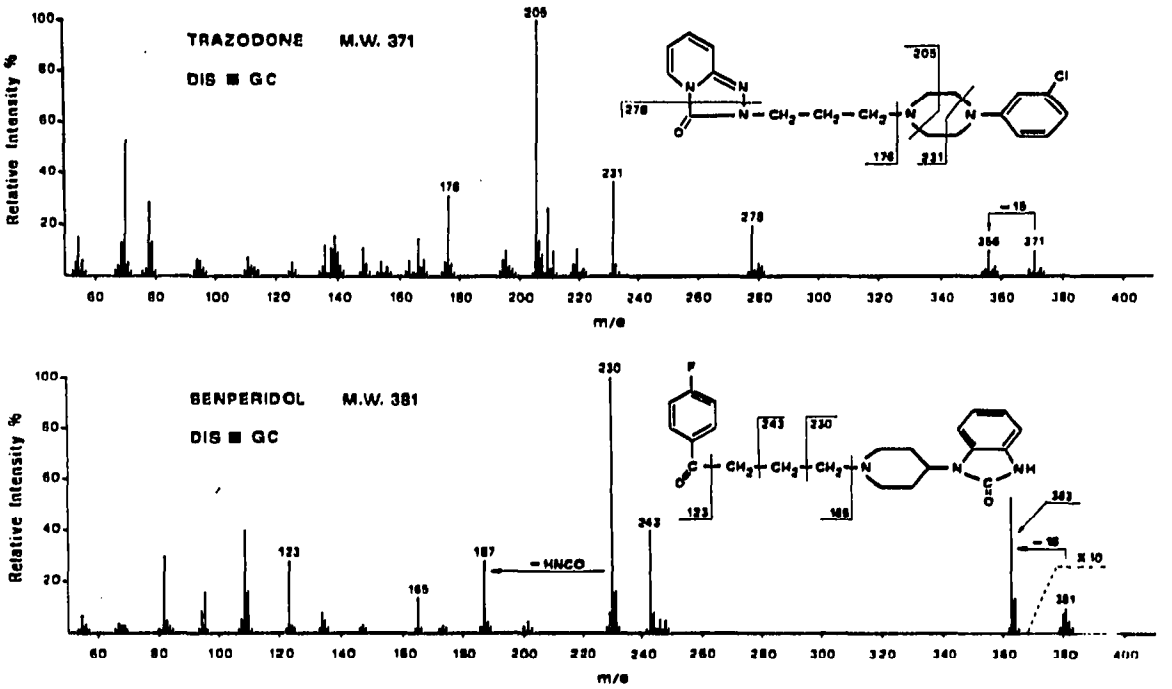

Fig. 2. Mass spectra of trazodone and benperidol obtained by the direct inlet system (DIS) and by the GC procedure.

The aqueous phase was extracted a second time with a further 7 ml of diethyl ether, and 7 ml of the ether extract was removed and added to the dry residue from the first extraction. After mixing, 2 ml of 0.1 *N* hydrochloric acid were added and the capped tubes shaken vigorously for 20 min and centrifuged for 5 min at 2000 *g* and 4°. A 1.9-ml volume of the acidic aqueous phase was then transferred into a third test-tube and washed once with 5 ml of chloroform and three times with 5 ml of diethyl ether. After discarding the organic phase, 0.1 ml of 6 *N* sodium hydroxide solution

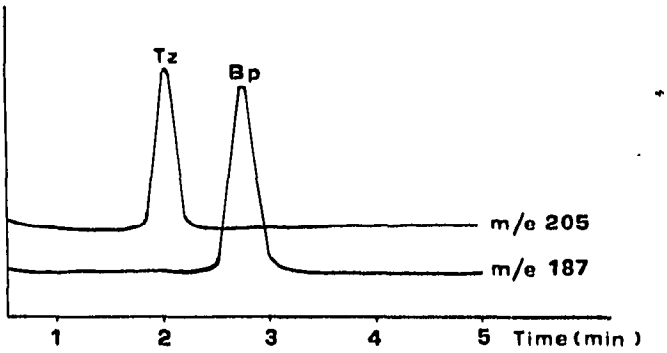

Fig. 3. Mass fragmentogram of trazodone (Tz) and the internal standard benperidol (Bp) obtained from biological extracts.

was added to the acidic aqueous phase and the mixture extracted twice with 7 ml of diethyl ether.

After centrifugation, the combined ether extracts were transferred into a fourth test-tube and evaporated to dryness under a gentle stream of nitrogen in a water-bath at 40°. Then 50 μl of a methanolic solution of benperidol (containing 1 or 10 μg of the drug, depending on the concentration range and the detector utilized) were added to the dry residue. The tubes were capped, shaken for 20 sec on a mixer and 1 or 2 μl were injected on to the GC column.

The extraction recoveries of trazodone from water and plasma were similar (85 $\pm$ 2%).

Kinetic parameters

These parameters were obtained by means of the BMD-X-85 program[11] on a PDP 11/45 digital computer.

RESULTS AND DISCUSSION

The gas chromatograms of trazodone and the internal standard obtained from water and plasma samples are shown in Fig. 1. The identities of the peaks were checked by means of GC–mass spectrometry and the mass spectra are reported in Fig. 2.

Fig. 3, showing the mass fragmentogram of trazodone and benperidol obtained from water and plasma extracts, indicates that no interference from endogenous substrates was found.

Internal calibration graphs (Fig. 4) were obtained by adding trazodone to water in amounts from 25 ng/ml to 50 μg/ml and plasma, and then processing the samples as described above.

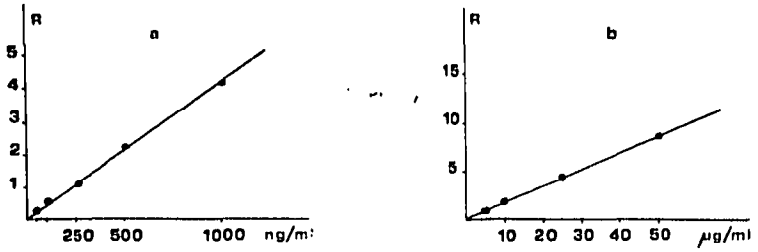

Fig. 4. Internal calibration graphs for water and plasma extracts (only one graph is reported because of the same recovery of extraction) obtained (a) by mass fragmentography and (b) by gas chromatography. R = ratio of the peak area of trazodone and the internal standard benperidol.

The linearity of the method ranges from concentrations of 25 ng/ml to 1 μg/ml when MF detection is used (the minimum detectable amount for injection being 200 pg) and from 1 to 50 μg/ml when the FID is used (the minimum detectable amount for injection being 20 ng).

By utilizing these GC and MF methods, the levels of trazodone in plasma were determined after a single administration of 10 mg/kg (i.v.) of the drug to CD_1 rats

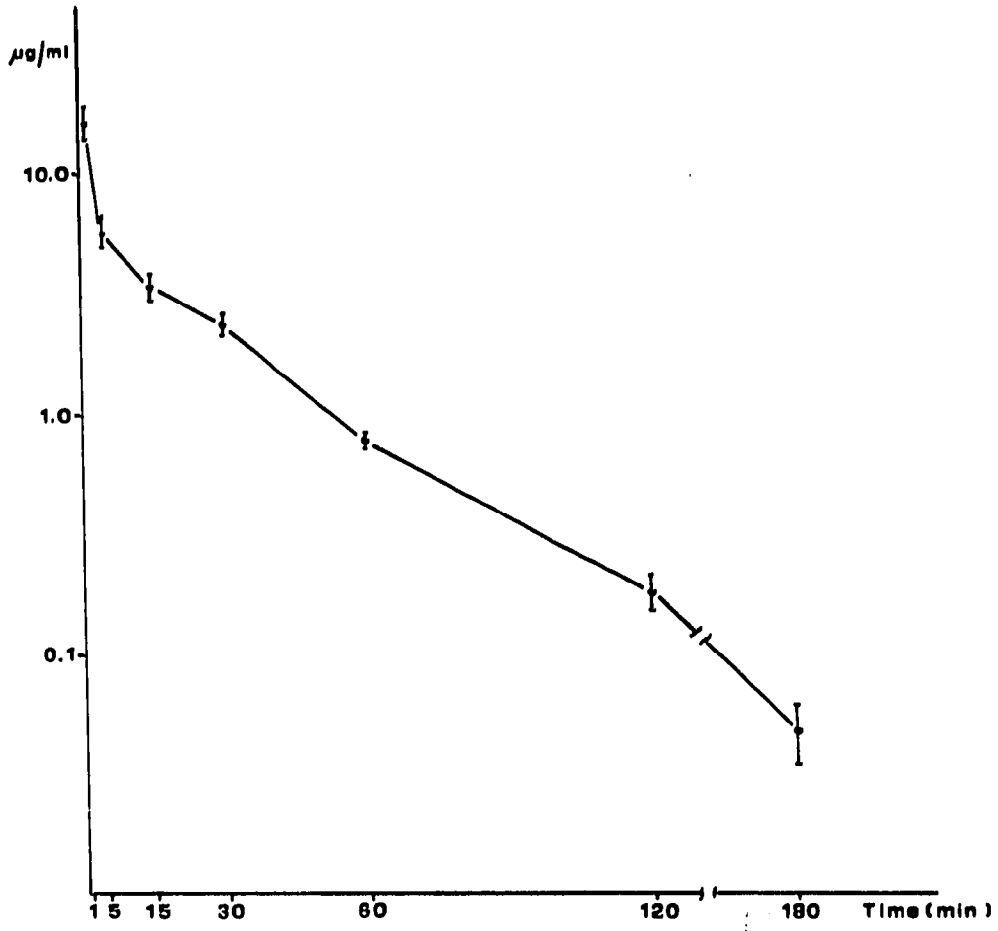

Fig. 5. Levels of trazodone in plasma at different times after intravenous injection. Each point is the average of three determinations. Measurements at 1, 5, 15 and 30 min were performed by using a flame ionization detector, while measurements at 60, 120 and 180 min were performed by using the mass fragmentographic technique. The pharmacokinetic parameters for plasma were: half-life (α) $t_{\frac{1}{2}}$, 1.2 min; (β) $t_{\frac{1}{2}}$, 22.9 min; (α) V_d, 0.489 l/kg; (β) V_d, 1.9 l/kg; area under the curve, $\int_0^{120} C_p dt$, 215 min·μg/ml; and total body clearance, 0.048 l/min/kg.

(average weight 350 g). The disappearance curve of trazodone in plasma and the pharmacokinetic parameters are reported in Fig. 5. It can be seen that trazodone disappears rapidly from the rat plasma, following a biphasic curve.

The specificity and sensitivity of the methods described, particularly that involving the use of the mass spectrometer as a GC detector, appear to be satisfactory for pharmacokinetic studies in humans and in animals after acute or repeated treatment with trazodone.

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