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Note

Simple and sensitive gas chromatographic method for the determination of camazepam in human plasma

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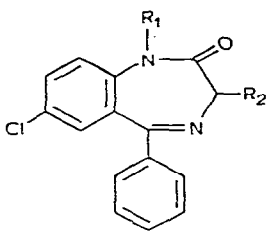
Camazepam (CZ) or 3-N,N-dimethylcarbamoyloxy-7-chloro-5-phenyl-1-methyl-1,3-dihydro-2H-1,4-benzodiazepine-2-one (Bristol Laboratories, Paris, France) is a new compound with a structure similar to that of oxazepam (OZ) and temazepam (TZ), which is a potent tranquillizer[1–5].



	R_1	R_2
OZ:	—H	—OH
TZ:	$-CH_3$	—OH
CZ:	$-CH_3$	$-OCON(CH_3)_2$

The aim of this work was to develop a technique for the measurement of plasma concentrations of CZ suitable for the determination of its pharmacokinetics in humans.

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EXPERIMENTAL

Standards and reagents

Camazepam and penfluridol [(chloro-4-α,α,α-trifluoromethyl-*m*-tolyl)-4-bis(*p*-fluorophenyl)-4,4-butyl-1-piperidinol-4], used as an internal standard (IS), were obtained from Bristol Laboratories.

Acetone and diethyl ether (Riedel de Haën, Seelze-Hannover, G.F.R.) were distilled before use.

The buffer solution, consisting of 1 *M* potassium dihydrogen orthophosphate solution (Merck, Darmstadt, G.F.R.), was made up in doubly-distilled water.

The stock solutions (1 mg/ml) of CZ and the IS were prepared in acetone and were stable for 6 months at 4°.

Working solutions were obtained by diluting the stock solutions in acetone to give concentrations of 25–500 ng/ml for CZ and 1 μg/ml for the IS.

Apparatus

A Hewlett-Packard Model 5710A gas chromatograph equipped with a ^{63}Ni electron-capture detector was used. The glass column (1 m × 3 mm I.D.) was packed with 3% OV-17 on Chromosorb W HP (80–100 mesh) (Pierce, Rockford, Ill., U.S.A.) and conditioned for 24 h at 280° (9:1 argon–methane, flow-rate 40 ml/min). The column temperature was 260°, injection port temperature 300°, detector temperature 300°, carrier-gas (9:1 argon–methane) flow-rate 80 ml/min, electrometer attenuation setting × 64–× 16 and recorder chart speed 5 mm/min.

For mass spectrometry, a VG Micromass 305F mass spectrometer combined with a Hewlett-Packard Model 5710A gas chromatograph and a VG Data System 2000 computer (M8 2R) were used. The electron energy was 70 eV, electron beam current 200 μA and temperature of ion source 240°. The column (glass, 2 m × 2 mm I.D.) was packed with 3% OV-17 on Chromosorb W HP (80–100 mesh) and operated at 280° with a helium flow-rate of 30 ml/min.

Extraction procedure

A 0.4-ml volume of IS solution (1 μg/ml) was introduced into a 15-ml glass-stoppered test-tube, and the solvent (acetone) was evaporated to dryness by passing a gentle stream of nitrogen into the test-tube in a 35° water-bath. Then, 1 ml of plasma, 2 ml of buffer solution (previously adjusted to pH 7.2 with 5 N potassium hydroxide solution and 8 ml of diethyl ether were added. The mixture was shaken for 15 min on a rotating mixer (60 rpm) and then centrifuged at 4000 rpm (1800 *g*) for 15 min at 4°. A 6-ml volume of the organic layer was transferred into a conical test-tube by passing through a small column (4 mm I.D.) filled with glass-wool to a height of 2 cm. The extraction procedure was repeated with 8 ml of diethyl ether and, after centrifugation, an 8-ml volume of the organic layer was added to the first 6 ml. The solvent was evaporated to dryness under the conditions already described. A 50-μl volume of acetone was added to the extract and 2–3 μl of this solution were injected into the GC column.

Calibration graph

Using the procedure described above, a calibration graph was obtained by

running plasma samples spiked with CZ at concentrations varying from 10 to 200 ng/ml and with IS at a fixed concentration of 400 ng/ml.

RESULTS AND DISCUSSION

Typical chromatograms obtained from normal plasma, plasma spiked with CZ (100 ng/ml) and IS (400 ng/ml) and plasma from a patient treated with CZ are shown in Fig. 1. The retention times were 10.7 and 17.0 min for CZ and IS, respectively.

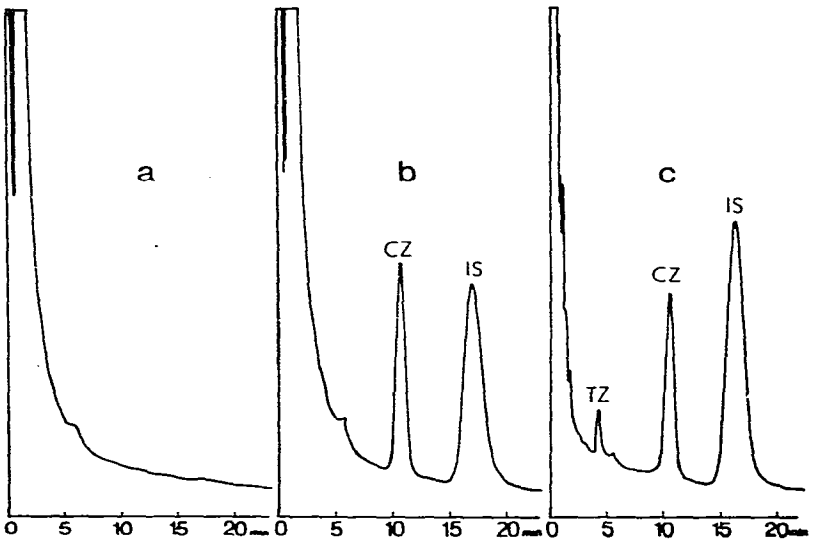

Fig. 1. Gas chromatograms of extracts from (a) normal plasma, (b) plasma spiked with 100 ng/ml of CZ and 400 ng/ml of penfluridol (IS) and (c) plasma from a patient receiving CZ. In the last instance TZ, a metabolite of CZ, is observed.

The identity of the peaks was checked by gas chromatography–mass spectrometry (GC–MS) and the analysis confirmed that the peaks were intact CZ and IS (Fig. 2).

The calibration graph for extracted CZ obtained by plotting the ratio of the peak area of CZ to that of IS against known amounts of CZ, is shown in Fig. 3. The graph was linear in the range of concentrations studied. The minimum detectable amount of CZ was 1 ng/ml.

The reproducibility was checked by analysing samples of plasma spiked with several concentrations of CZ. The results are shown in Table I. The precision of the method was 3.7%.

No interference from endogenous substances was noted. Other tranquillizers (TZ and OZ, possible metabolites of camazepam) were studied; these two compounds exhibit peaks at retention times shorter than that of CZ (4.3 and 1.5 min for TZ and OZ, respectively).

The GC–MS analysis (Fig. 4) showed that the OZ peak observed at 1.5 min

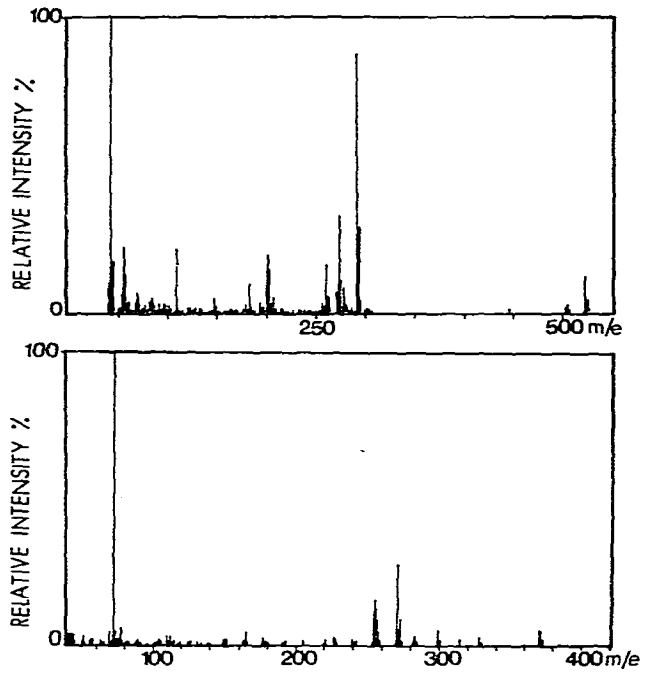

Fig. 2. Mass spectra obtained during the GC–MS analysis for penfluridol (IS) and CZ.

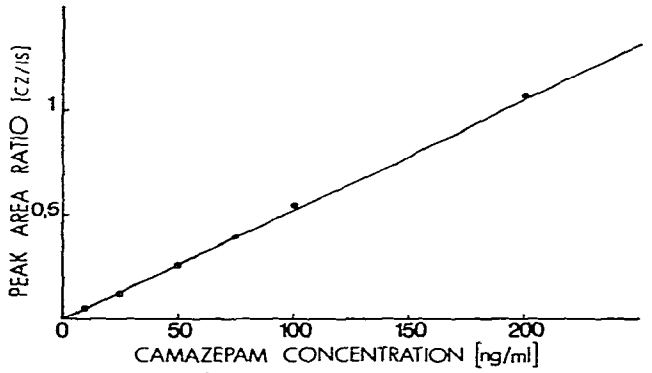

Fig. 3. Calibration graph for CZ with IS.

TABLE I

RECOVERY OF ADDED CAMAZEPAM FROM PLASMA

Added (ng/ml)	*Found (±SEM)* (ng/ml)*	*Number of trials*
10	9.1 ± 0.19	8
20	19.0 ± 1.16	8
40	36.9 ± 0.54	8
100	98.1 ± 2.51	7

* SEM = standard error of the mean.

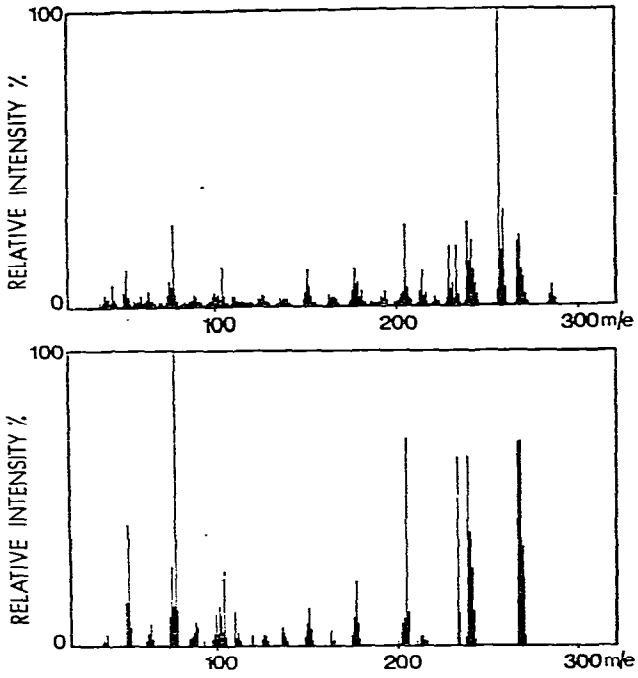

Fig. 4. Mass spectra obtained by direct introduction (top) and during the GC–MS analysis for OZ.

did not correspond to the intact benzodiazepine, but rather to the quinazoline carboxaldehyde derivative formed by on-column rearrangement[6–10]. A similar degradation was found with TZ and the peak at 4.3 min corresponded to the derivative formed under the same conditions.

As shown in Fig. 1, TZ is present in only very small amounts in the plasma from a patient treated with CZ.

Fig. 5 shows that the method allowed the determination of the plasma con-

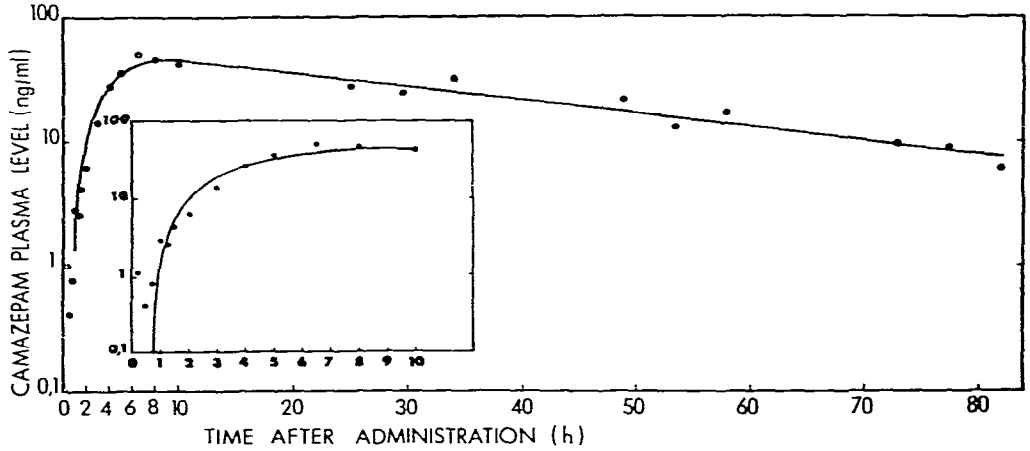

Fig. 5. Plasma levels of camazepam in a healthy volunteer after a single oral administration of 20 mg. The inset shows the fitting on an expanded time scale.

centration-time curve [$C = f(t)$] after a single oral administration of 20 mg of CZ to a healthy volunteer. The approximate values of the parameters of this equation were determined by using the peeling method. It appeared that the equation was the sum of three exponentials:

$$C = -A_1e^{-k_at} + A_2e^{-\alpha t} + A_3e^{-\beta t}$$

In order to fit the most probable curve, several iteration programs, based upon the least-squares method, were devized for a digital computer. The best fit was obtained for a two-compartment linear model in which two macro-constants were identical according to a resonance phenomenon[11]. Under these conditions the equation of the most probable curve corresponding to the experimental data was

$$C = -(55.57 + 46.97t)e^{-0.553t} + 61.13e^{-0.025t}$$

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