Journal of Chromatography, 166 (1978) 604–608

CHROM. 11,383

Note

Rapid gas–liquid chromatographic determination of chlordiazepoxide in serum

SY-RONG SUN

Pharmaceutical Products Division, Abbott Laboratories, North Chicago, Ill. 60064 (U.S.A.)

(Received July 7th, 1978)

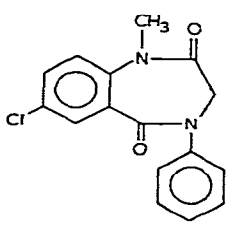
Electron-capture gas chromatographic (GC) methods for determining chlordiazepoxide in biological samples by assaying either the hydrolysis product[1,2] or the intact moiety[3,4] have been reported earlier. Zingales[3] used the less polar solvent heptane to extract chlordiazepoixde from alkalinized plasma, and the drug was re-extracted into acid and then back extracted again into small volumes of heptane when analyzing samples at low concentrations. It was reported that attempts to concentrate the drug in the heptane extract by evaporation of the solvent resulted in contamination of the electron-capture detector and loss of sensitivity to such an extent that no chlordiazepoxide could be detected in samples.

However, by using a modified procedure, low drug levels could be analyzed after concentration of heptane extract by evaporation without contamination of the detector. Modifications were made in the use of a ^{63}Ni constant-current elctron-capture detector, and a different column and extraction procedure. An internal standard was used in our procedure to compensate for losses during extraction and variability in the injection amount to enhance reproducibility. The present procedure is rapid, quantitative, and readily automated, and the sensitivity and specificity fulfill the requirements for its use in clinical pharmacokinetic evaluation after a single dose of chlordiazepoxide.

EXPERIMENTAL

Reagents and materials

All reagents were of analytical grade and chlordiazepoxide hydrochloride (7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide hydrochloride) and internal standard [7-chloro-1-methyl-4-phenyl-1*H*-1,4-benzodiazepine-2,5-(3*H*, 4*H*)-dione] were used as supplied.



Internal standard

Instrumentation

A Hewlett-Packard Model 5830A reporting gas–liquid chromatograph equipped with a Model 18850A computing integrator and Model 18803A ^{63}Ni constant-current electron-capture detector was used. The column was 1.21 m (4 ft.) × 4 mm I.D., glass, packed with 10% OV-1 on Chromosorb G HP (80–100 mesh). The column was conditioned at 275° for at least 18 h with an argon–methane (95:5) carrier gas flowing through at 50 ml/min. The oven column temperature was 270°, the injection port 270°, and the detector temperature 325°. The attenuation was 2^7 and slope sensitivity was 1.1 mV/min. Under these conditions, the internal standard and chlordiazepoxide have retention times of 4.95 and 6.99 min, respectively (Fig. 1).

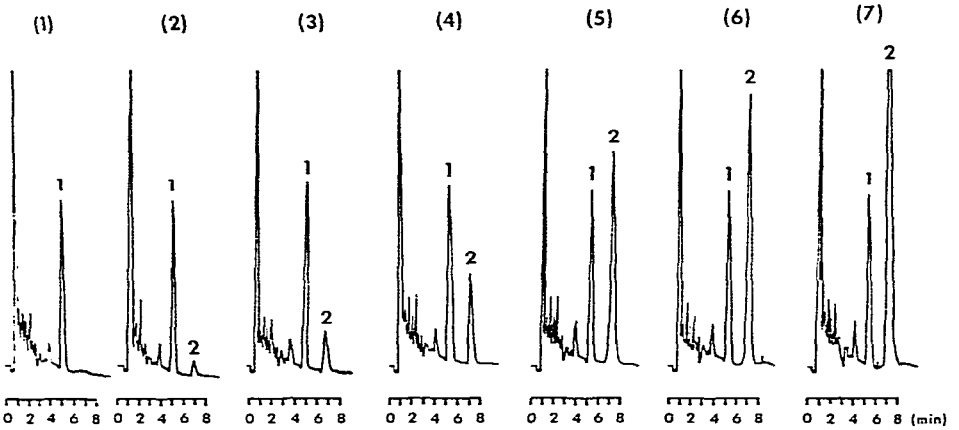


Fig. 1. Typical GC tracings of chlordiazepoxide assay in serum. Chromatograms: (1) Serum blank with internal standard; (2) chlordiazepoxide serum standard at 0.1 μg/ml serum; (3) chlordiazepoxide serum standard at 0.2 μg/ml serum; (4) chlordiazepoxide serum standard at 0.3 μg/ml serum; (5) chlordiazepoxide serum standard at 0.5 μg/ml serum; (6) chlordiazepoxide serum standard at 0.7 μg/ml serum; (7) chlordiazepoxide serum standard at 1.0 μg/ml serum. Peaks: 1 = internal standard; 2 = chlordiazepoxide.

Preparation of standards

Serum standards at 0, 0.1, 0.2, 0.3, 0.5, 0.7, 1.0, 1.5 and 2.0 μg/ml were used for the study. A serum standard at 2.0 μg/ml was prepared by diluting 2 ml of a primary standard (100 μg/ml water) with serum to 100 ml. An internal standatd solution at 0.4 μg/ml water was prepared from a primary standard at 100 μg/ml methanol. The serum standards were extracted along with the unknown samples.

Assay procedure

To 1 ml serum sample in a 20-ml screw-capped test tube were added 1 ml of internal standard solution (0.4 μg/ml water), 1 ml 0.1 *N* sodium hydroxide and 10 ml heptane–isoamyl alcohol (98.5:1.5). After shaking at 80 rpm on an Eberbach shaker for 10 min and centrifugation at 1700 *g* at 10° for 5 min, 9 ml of the organic layer (upper layer) was transferred into a conical tube and dried at 50° with filtered air to dryness. The residue was reconstituted with 1 ml of benzene by vortexing and transferred to a disposable GC vial using a disposable pipette. After capping the vial, a 10-μl aliquot was injected into the GC column by an automatic sampler.

Calibration curve

A calibration curve was constructed by plotting peak area ratio (chloridazepoxide/internal standard) against the chlordiazepoxide concentration in serum. A non-linear curve (Fig. 2) was obtained and the unknown concentrations were read from this curve.

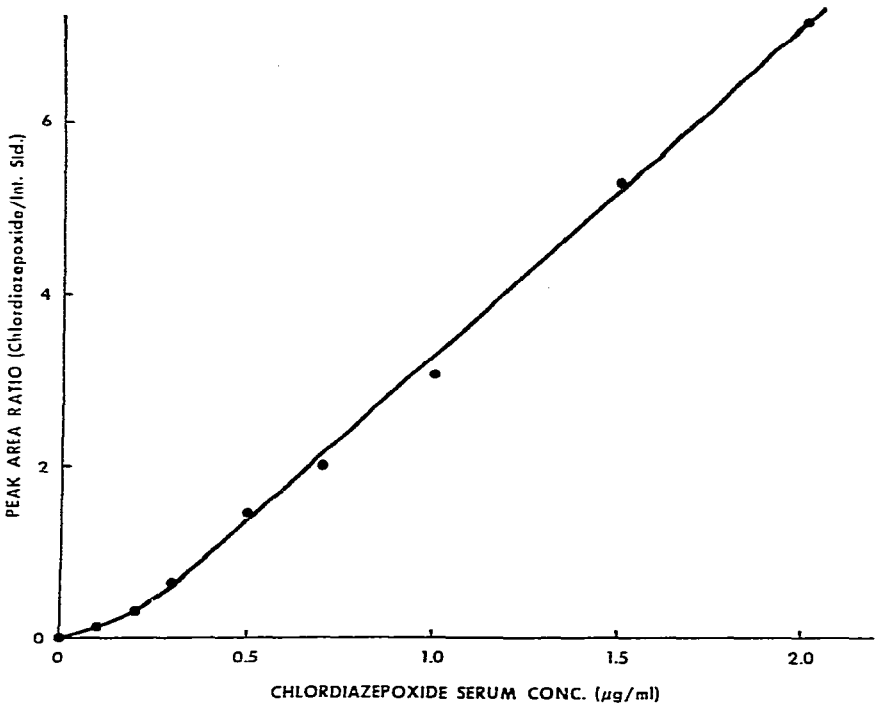


Fig. 2. Calibration curve of chlordiazepoxide assay.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms of extracts from 1 ml of serum spiked with chlordiazepoxide and internal standard. Peak shapes were good. Chlordiazepoxide and the internal standard were well resolved from co-extractive peaks. Under the assay conditions, the retention times for demoxepam and desmethylchlordiazepoxide, the two known metabolites of the drug[5,6], were 2.39 and 2.46 min, respectively. Therefore, the assay is specific for the parent drug.

The reproducibility of the assay is shown in Table I. Relative standard deviations ranged from 3.3 to 10.8% for serum concentrations from 0.1 to 1.0 μg/ml. A typical serum concentration *versus* time curve for one subject is shown in Fig. 3. Peak serum chlordiazepoxide concentration occurred at 1 h and was 1.0 μg/ml.

This modified assay method is rapid and precise and could be used to conduct bioavailability studies in humans.

TABLE I

ASSAY REPRODUCIBILITY

Chlordiazepoxide serum concentration (μg/ml)	*Calculated serum concentration* (μg/ml)*	*Relative standard deviation (%)*
0	0	—
0.1	0.087 ± 0.0058	6.6
0.25	0.213 ± 0.0231	10.8
0.50	0.500 ± 0.0529	10.6
1.00	0.923 ± 0.0306	3.3

* Mean ± standard deviation of three determinations at each concentration.

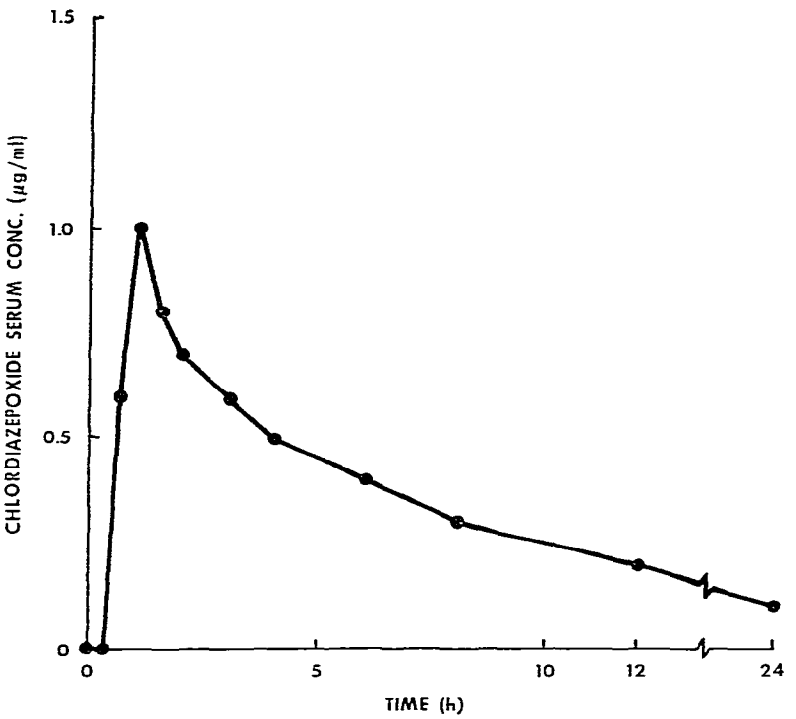


Fig. 3. Serum levels of chlordiazepoxide of one individual after receiving a 20-mg oral dose of chlordiazepoxide·HCl.

ACKNOWLEDGEMENTS

Technical assistance from G. Thomas and helpful discussions with Dr. D. Hoffman are gratefully acknowledged.

REFERENCES

1 J. A. F. de Silva, M. A. Schwartz, V. Stefanovic, J. Kaplan and L. D'Arconte, *Anal. Chem.*, 36 (1964) 2099.

2 J. A. F. de Silva, B. A. Koechlin and G. Bader, *J. Pharm. Sci.*, 55 (1966) 692.
3 I. A. Zingales, *J. Chromatogr.*, 61 (1971) 237.
4 J. A. F. de Silva, I. Bekersky, C. V. Puglisi, M. A. Brooks and R. E. Weinfeld, *Anal. Chem.*, 48 (1976) 10.
5 M. A. Schwartz, E. Postma and Z. Gaut, *J. Pharm. Sci.*, 60 (1971) 1500.
6 S. Sun, *Abstracts, Amer. Pharm. Assoc. Annual Meeting, New York*, 7 (1977) 194; *J. Pharm. Sci.*, 67 (1978) 639.