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Note

Plasma concentrations of temazepam, a 3-hydroxy benzodiazepine, determined by electron-capture gas—liquid chromatography

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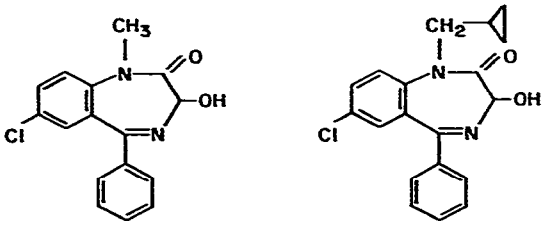
Temazepam (Fig. 1) is a 1,4-benzodiazepine used clinically for the treatment of insomnia [1—3]. Temazepam is biotransformed in humans by glucuronide conjugation of the 3-hydroxy substituent, yielding a water-soluble glucuronide metabolite excreted in urine [4]. This paper describes a gas—liquid chromatographic method for the analysis of temazepam in human plasma following therapeutic doses.



TEMAZEPAM *3-HYDROXY-PRAZEPAM*

Fig. 1. Structures of temazepam and its internal standard, 3-hydroxy-prazepam.

EXPERIMENTAL AND RESULTS

Apparatus and chromatographic conditions

The instrument used is a Hewlett-Packard Model 5750 gas chromatograph equipped with a 2-mCi ^{63}Ni electron-capture detector operated in the pulsed

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mode with a pulse interval of 150 μsec. The column is coiled glass, 183 cm × 2 mm I.D., packed with 3% SP-2250 on Supelcoport (80—100 mesh). The carrier gas is ultrapure helium at a flow-rate of 50 ml/min. The purge gas is argon—methane (95:5), at a flow-rate of 80 ml/min. Operating temperatures are: injection port, 310—320°C; column, 280°C; detector, 320°C. The column is primed daily by an injection of 2—3 μl of a solution of azolectin dissolved in benzene (1 mg/ml).

Stock solutions

Ten milligrams of temazepam (TMZ) kindly supplied by Sandoz (East Hanover, NJ, U.S.A.) and of its structural analog, 3-hydroxy-prazepam (3-OH-PRZ) kindly supplied by Warner-Lambert (Morris Plains, NJ, U.S.A.) (Fig. 1) are each dissolved in 2—3 ml of absolute ethanol, then diluted to 100 ml with benzene. The stock solutions, containing 100 μg/ml, are stored in amber-colored bottles at 40°C. Working standards containing 1 μg/ml of TMZ and 5 μg/ml of 3-OH-PRZ are prepared as needed by appropriate dilution with benzene.

Preparation of samples

3-OH-PRZ serves as the internal standard. A constant amount (250 ng) is added to a series of 13-ml round bottom glass culture tubes equipped with PTFE-lined screw-top caps. Calibration standards are prepared by adding TMZ from the working standard solution in the following amounts: 25, 50, 75, 100, 200, and 300 (or 400) ng. The contents of both the calibration and sample tubes are evaporated to dryness at 40—50°C under mildly reduced pressure.

One ml of drug-free control plasma is added to each of the calibration tubes, and 1 ml of unknown plasma is added to each of the sample tubes which contain only internal standard. Calibration standards are extracted and analyzed daily together with each set of unknowns. No buffering or other sample preparation is required. When plasma TMZ concentrations exceeding 300—400 ng/ml are anticipated, plasma aliquots of less than 1 ml can be analyzed. The final volume is adjusted to 1.0 ml by addition of distilled water.

Extraction

After addition of 3—5 ml of benzene (containing 1.5% isoamyl alcohol), the tubes are agitated gently in the upright position on a vortex-type mixer for 60 sec, then centrifuged at room temperature for 10 min at 400 *g*. An aliquot of the organic layer is transferred to another tube, and the procedure is repeated. The combined organic extracts are evaporated to dryness at 40°C under mildly reduced pressure. The residue is redissolved in 50—100 μl of benzene (containing 15% isoamyl alcohol), of which 1—3 μl is injected into the chromatograph.

Evaluation of the method

Under the described chromatographic conditions, approximate retention times for TMZ and 3-OH-PRZ are 4 min and 6 min, respectively (Fig. 2).

A linear relationship exists between concentrations of TMZ in the calibration standards and the peak height ratio of TMZ to its internal standard. The day-to-

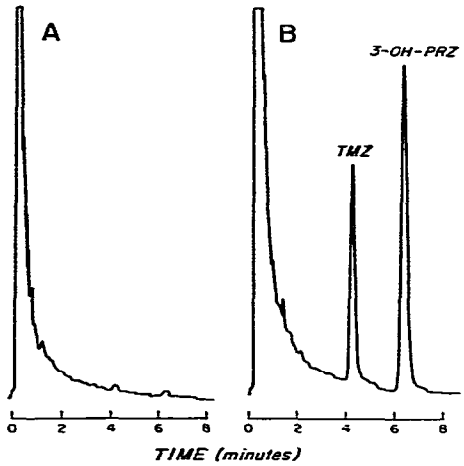

Fig. 2. (A) Chromatogram of a drug-free control plasma extract; (B) the same sample to which was added temazepam (TMZ), 100 ng/ml, and 3-hydroxy-prazepam (3-OH-PRZ), 250 ng/ml.

day coefficient of variation in the slope of the calibration curves was 6.9% ($n = 18$).

The sensitivity limits are approximately 5 ng of TMZ per ml of original sample. Coefficients of variation for identical samples ($n = 8$ at each concentration) were 5.4% at 25 ng/ml, 5.5% at 50 ng/ml, and 8.7% at 200 ng/ml. The mean deviation between 42 randomly selected duplicate samples was 6.2%. As in the case of other benzodiazepines [5, 6], recovery of TMZ and 3-OH-PRZ is more than 95%.

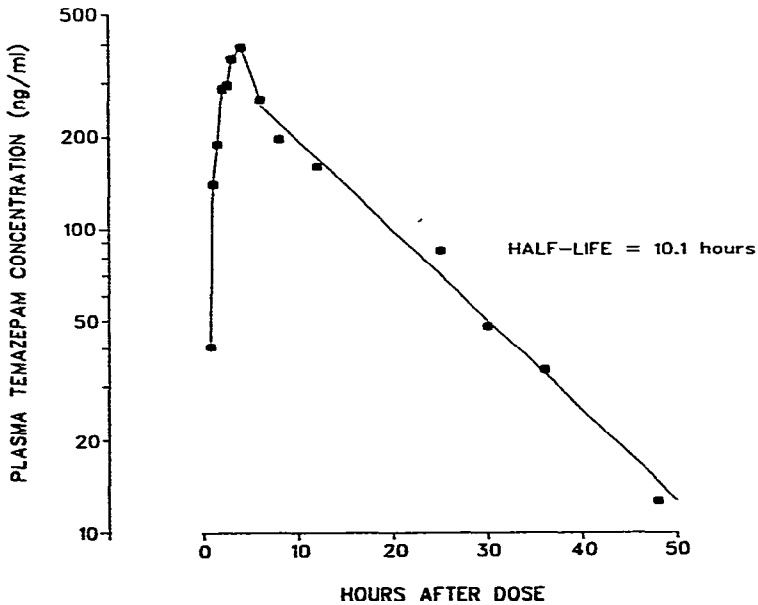

Fig. 3. Plasma temazepam concentrations for 48 h after a single 30-mg dose administered to a healthy volunteer.

Clinical pharmacokinetic study

A healthy 29-year-old female participated after giving informed consent. A single 30-mg dose of TMZ (Restoril^{R}, Sandoz) was administered with 100 ml of water after an overnight fast. Venous blood samples were drawn into heparinized tubes at multiple points in time over 48 h. Plasma was separated and frozen until assay. Plasma concentrations of TMZ were determined as described above.

A peak TMZ concentration of 392 ng/ml was measured in the sample drawn 3.0 h after the dose. Elimination proceeded with a half-life of 10.1 h (Fig. 3).

CONCLUSION

This paper describes a rapid and sensitive method for the quantitation of TMZ in human plasma. Both TMZ and 3-OH-PRZ are extracted into the organic solvent at physiologic pH using a double extraction technique. The organic solvent is then evaporated, and the redissolved residue is injected directly into the chromatograph. Plasma samples are consistently free of contaminants in the areas corresponding to retention times of TMZ and 3-OH-PRZ, making extensive cleanup unnecessary. The underivatized compounds produce symmetric, gaussian peaks under the described conditions largely due to the excellent performance of the 50:50 phenyl methyl silicone liquid phase (SP-2250) that was utilized.

Application of the method to pharmacokinetic studies of TMZ in humans is illustrated. Consistent with previous reports [7], the elimination half-life of TMZ appears to fall in the short to intermediate range and is similar to half-life values reported for oxazepam and lorazepam, two other 3-hydroxybenzodiazepines in wide clinical use [8, 9].

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