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## Note

# Electron-capture gas chromatographic analysis of the triazolobenzodiazepines alprazolam and triazolam

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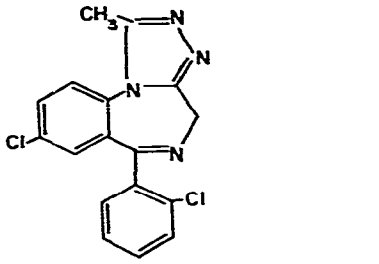
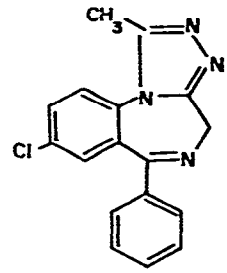
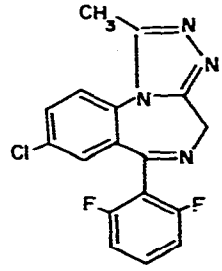
Alprazolam and triazolam (Fig. 1) are triazolobenzodiazepine derivatives used clinically as anxiolytic and hypnotic agents, respectively [1, 2]. Because




TRIAZOLAM




ALPRAZOLAM




U-31485

Fig. 1. Structural formulae of the triazolobenzodiazepines, triazolam and alprazolam. Also shown is the structure of U-31485, the analogue used as internal standard in the analysis.

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of their high milligram potency, therapeutic doses of these two agents are low and concentrations in plasma are correspondingly low. Currently available pharmacokinetic data on alprazolam and triazolam have been derived from studies of the radiolabeled drugs [3—5] or with use of high-performance liquid chromatography [6]. This paper describes an electron-capture gas chromatographic (GC) assay of alprazolam and triazolam that can be utilized for quantitation of these two compounds in human plasma following single therapeutic doses.

## EXPERIMENTAL

### *Instrumentation*

The analytic instrument is a Hewlett-Packard Model 5750 gas chromatograph equipped with a 2-mCi electron-capture detector operated in the pulsed mode with a pulse interval of 150 μsec. The column is coiled glass, 1.83 m × 2 mm I.D., packed with 1% OV-17 on 80—100 Chromosorb W HP (Hewlett-Packard, Avondale, PA, U.S.A.). The carrier gas is ultrapure helium, at a flow-rate of 50 ml/min. The detector purge gas is argon—methane (95:5), at a flow-rate of 80 ml/min. Operating temperatures are: column, 290°C; detector and injection port, 310°C. The column is primed prior to each day's use by injection of 2—3 μl of a solution of purified soy phosphatides in benzene (1 mg/ml) (Asolectin, Associated Concentrates, Woodside, NY, U.S.A.).

### *Reagents and standards*

Stock solutions are prepared by dissolving 10 mg each of triazolam, alprazolam, and of U-31485, a triazolobenzodiazepine analogue used as internal standard (Fig. 1), in a small amount of ethanol. The volume is made to 100 ml with benzene or toluene. Working standards containing 0.1 μg/ml of each compound are prepared by appropriate dilution with benzene or toluene. Stock solutions and working standards are stable for at least two months when stored in amber bottles at 4°C.

### *Preparation and extraction of samples*

To a series of 13-ml round-bottom culture tubes equipped with PTFE-lined screw top caps is added 10 ng of the internal standard (100 μl of working standard solution). The solvent is evaporated to dryness at 40—50°C under mildly reduced pressure. A series of calibration tubes is prepared by adding 1, 2.5, 5, 7.5, 10, and 12.5 ng of alprazolam or triazolam to a series of these tubes. The solvent again is evaporated to dryness under mildly reduced pressure. A 0.5—1.0 ml aliquot of drug-free control plasma is added to each of the calibration tubes; 0.5—2.0 ml of unknown plasma is added to all of the other tubes. No other sample preparation is necessary.

To each tube are then added 3 ml of benzene (containing 1.5% isoamyl alcohol), and the tubes are agitated gently in the upright position in a Vortex-type mixer. The samples are centrifuged, and an aliquot of the organic phase is transferred to a tapered centrifuge tube. The solvent is evaporated to dryness at 40—50°C under mildly reduced pressure. The residue is redissolved in 25 μl of toluene (containing 15% isoamyl alcohol), of which 3—6 μl are injected onto the chromatograph.

*Clinical pharmacokinetic study*

A healthy 39-year-old volunteer participated in two clinical pharmacokinetic studies. On one occasion, he ingested a single 1.0-mg dose of alprazolam (two 0.5-mg tablets) in the fasting state. Multiple venous blood samples were drawn during the next 48 h. The samples were centrifuged, and the plasma separated and frozen until the time of assay. On another occasion, he ingested a single 0.5-mg dose of triazolam (two 0.25-mg tablets) in the fasting state. Multiple samples were drawn over the next 12 h. The plasma was separated and frozen until the time of assay. Concentrations of alprazolam and triazolam were determined using the method described above.

## RESULTS

*Evaluation of the method*

Under the described chromatographic conditions, approximate retention times are: U-31485, 2.2 min; alprazolam, 3.3 min; triazolam, 4.1 min (Fig. 2). The relationship between plasma concentration of either drug and the peak height ratio of the drug to the internal standard is linear up to concentrations of 15 ng/ml.

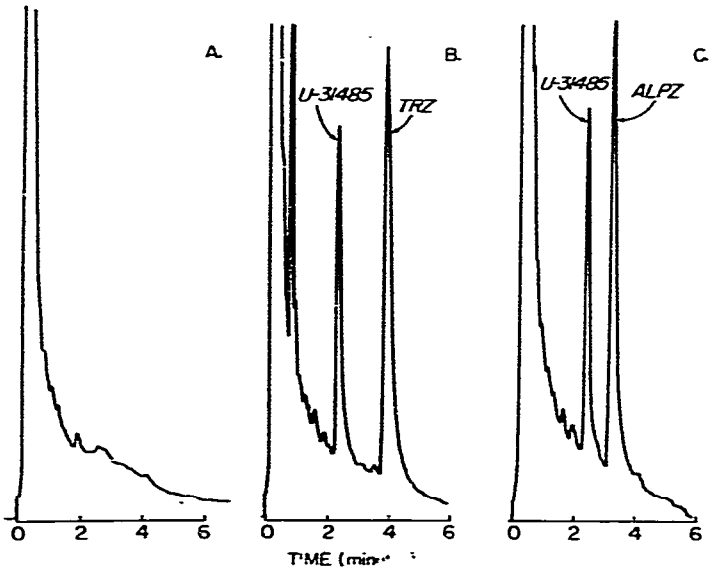


Fig. 2. (A) Chromatogram of an extract of 1 ml of drug-free control plasma sample. (B) Chromatogram of the same sample after addition of 10 ng each of triazolam (TRZ) and of the internal standard, U-31485. (C) Chromatogram after addition of 10 ng each of alprazolam (ALPZ) and of the internal standard. See text for chromatographic conditions.

The limit of sensitivity is approximately 0.25 ng of either compound per ml of plasma. Table I shows the replicability of identical samples at various concentrations. In all cases, the coefficient of variation was less than 6%. The mean deviation between 123 randomly selected replicate determinations of alprazolam was 3.9%. The between-day coefficient of variation in

TABLE I

REPLICABILITY OF IDENTICAL SAMPLES
$n = 6$ at each concentration.

| Plasma concentration (ng/ml) | Coefficient of variation (%) | |
|---|---|---|
| | Alprazolam | Triazolam |
| 1.0 | 4.2 | 5.3 |
| 2.5 | 4.7 | 3.7 |
| 5.0 | 5.8 | 3.2 |
| 10.0 | 3.6 | 2.5 |

the slope of consecutive standard curves was 5.2%. Residue analysis indicated that extraction of all three compounds from plasma is greater than 95% complete.

*Pharmacokinetic results*

After oral administration of alprazolam, a peak concentration of 22 ng/ml was measured in the sample drawn 1.0 h after dosage. Following attainment of the peak concentration, elimination proceeded thereafter with an apparent half-life of 13.2 h (Fig. 3). Administration of 0.5 mg of triazolam

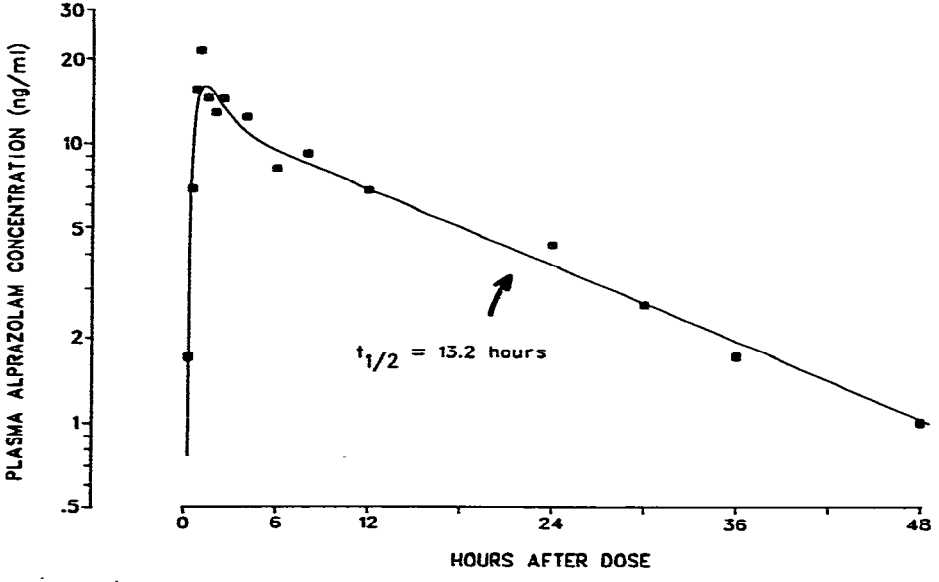


Fig. 3. Plasma alprazolam concentrations following a single 1.0-mg dose of alprazolam administered to a healthy volunteer. Solid line was determined by iterative nonlinear least-squares regression analysis using methods described previously [7].

to the same subject yielded a peak concentration of 14 ng/ml measured in the first sample drawn 0.25 h after the dose. Thereafter, the apparent elimination half-life was 3.1 h (Fig. 4).

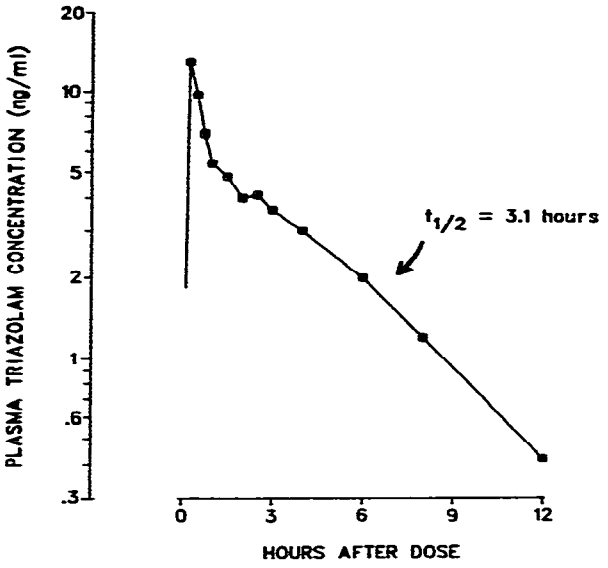


Fig. 4. Plasma triazolam concentrations following a single 0.5-mg oral dose administered to a healthy volunteer. Elimination half-life was determined by least-squares regression analysis of the terminal log—linear portion of the curve.

## DISCUSSION

This paper describes a rapid sensitive method for quantitation of alprazolam and triazolam in human plasma following single therapeutic doses. The methodology is made possible by the sensitivity of the electron-capture detector. Alprazolam, triazolam, and the internal standard all are essentially quantitatively extracted from plasma into an organic solvent at physiologic pH with no sample preparation. Since chromatograms of drug-free plasma samples are consistently free of interfering contaminant peaks, cleanup of samples is not necessary. Pharmacokinetic studies in humans utilizing radioactive alprazolam and triazolam [3—5] indicated that unconjugated metabolites of these compounds appeared in only negligible amounts in human plasma and, in any case, do not yield interfering chromatographic peaks.

Kinetic properties of alprazolam and triazolam were consistent with previous reports [3—6]. Peak plasma concentrations were reached shortly after a single oral dose, indicating reasonably rapid absorption from the gastrointestinal tract. Following attainment of peak levels, the apparent elimination half-life differed between the two drugs. Alprazolam was eliminated with a half-life of approximately 13 h. This drug is intended to serve as an anxiolytic or antidepressant agent, with a recommended twice or three times daily schedule of administration [1]. The half-life of triazolam, on the other hand, is considerably shorter. This compound is used clinically as a short-acting hypnotic agent [2]. Due to its short half-life, administration on a nightly basis would lead to essentially no drug accumulation. Further studies are needed to determine individual variability in the pharmacokinetic properties of these

two compounds, as well as disease states and drug interactions that might influence their disposition.

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