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Short Communication

Rapid and sensitive gas chromatographic determination of estazolam

J. F. Kelly and D. J. Greenblatt*

Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111 (USA)

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ABSTRACT

The triazolobenzodiazepine estazolam can be quantitated by gas chromatography with electron-capture detection. After addition of a suitable internal standard, plasma samples are extracted into toluene–isoamyl alcohol or benzene–isoamyl alcohol. The organic extract is separated, evaporated to dryness, reconstituted, and chromatographed using a 50:50 methyl–phenyl column (SP-2250). The sensitivity limit is approximately 1 ng of estazolam in a 1-ml sample. The method is suitable for clinical or experimental pharmacokinetic studies.

INTRODUCTION

Estazolam is a triazolobenzodiazepine derivative administered clinically as a hypnotic agent. Quantification of estazolam by gas chromatography with electron-capture detection [1], and by high-performance liquid chromatography [2–4], have been described previously. Here we report a simplified method whereby sensitivity and specificity are maintained but preparation time is reduced. The method is suitable for use in clinical or experimental pharmacokinetic studies.

EXPERIMENTAL

A gas chromatograph (Model 5840A, Hewlett-Packard, Palo Alto, CA, USA) equipped with an electron-capture detector was used. The column was coiled glass, 1.2 m × 4 mm I.D., packed with 3% SP-2250 on 80/100 Supelcoport (Supelco, Bellefonte, PA, USA). The carrier gas was a mixture of argon–methane (95:5), flow-rate 40–50 ml/min. Operating temperatures were: injection port and detector, 310°C; column oven, 280°C. Before being connected to the detector, a new column was conditioned at 290°C with a carrier gas flow-rate of 5–10 ml/min. At the start of each work day, the column was primed with 2–4 μl of purified soy phosphatides (Asolectin, Associated Concentrates, Woodside, NY, USA) in benzene (1 mg/ml).

The following reagents were used as received from commercial sources: benzene, isoamyl alcohol, toluene and poly(ethylene glycol). Pure estazolam was kindly provided by Takeda Chemical Industries (Osaka, Japan). The internal standard

* Corresponding author.

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(I.S.) U-31485 (8-chloro-6-(2,6-difluorophenyl)-1-methyl-4H-s-triazolo[4,3-a]-[1,4]benzodiazepine) [5], was provided by Upjohn (Kalamazoo, MI, USA). Stock solutions were prepared by dissolving 10 mg estazolam in 100 ml of toluene, and 10 mg I.S. in 100 ml of methanol. Working solutions were prepared by sequential dilution using the same solvents. The solutions were stored at 4°C.

A 50-μl volume of I.S. working solution (1 μg/ml), containing 50 ng of I.S., was added to a series of 13-ml round-bottom glass culture tubes with PTFE-lined screw-top caps. Calibration standards for estazolam were prepared by adding 1.0, 2.5, 5, 10, 25, 50, 75, 100, 125, 150, 175 and 200 ng of drug to consecutive tubes. The organic layer was evaporated to dryness at 40°C under moderately reduced pressure. A 1-ml sample of drug-free control serum or plasma was added to each of the calibration tubes; unknown plasma samples were added to tubes containing only I.S. A 2-ml volume of toluene–isoamyl alcohol or benzene–isoamyl alcohol (98.5:1.5) was added to each tube. Tubes were vortex-mixed in the upright position for 1 min and then centrifuged at room temperature for 5 min at 400 *g*. The organic layer was transferred to 2-ml autosampler vials and evaporated to dryness as described above. The samples were reconstituted with 250 μl of toluene–isoamyl alcohol–asolectin solution (84:13:3) of which 6 μl were injected into the chromatograph by the automatic injector. The asolectin solution was included to minimize column adsorption [6].

Male CD-1 mice, 6–8 weeks old, received a 5 mg/kg intraperitoneal dose of estazolam dissolved in poly(ethylene glycol). Animals were sacrificed at the following times after dosage: 0.5, 1, 2, 3, 4, 5 and 6 h. Trunk blood was collected into heparinized tubes, then centrifuged. Aliquots of plasma, with volumes ranging from 0.05 to 0.25 ml, were diluted with 1.5 ml of normal saline and extracted as described above.

Standard curves were prepared from the peak-height ratio (estazolam to I.S.) plotted *versus* known concentrations of estazolam. Mouse plasma concentrations were determined from the slope of the standard curve using linear regression analysis, with appropriate correction for the volume of the original plasma aliquot.

RESULTS AND DISCUSSION

Under the described conditions, the retention times for the I.S. and estazolam were approximately 5.5 and 7.0 min, respectively (Fig. 1). These values will vary depending on the characteristics of the specific column as well as the age of the column. Extracts of blank plasma contained no interfering peaks. The relation between estazolam concentration and peak-height ratio (*versus* I.S.) was linear to at least 200 ng/ml. The within-run coefficient of variation for identical samples ($n = 4$) was 15.4% at 1.0 ng/ml, 10.2% at 2.5 ng/ml, and less than 4% for concentrations of 5 ng/ml and higher. The between-run coefficient of variation in the slope of the calibration curves was 1.6%. The limit of detection was 1 ng/ml for a 1-ml sample.

Fig. 2 illustrates the plasma concentration–time curve for CD-1 mice given a single 5 mg/kg

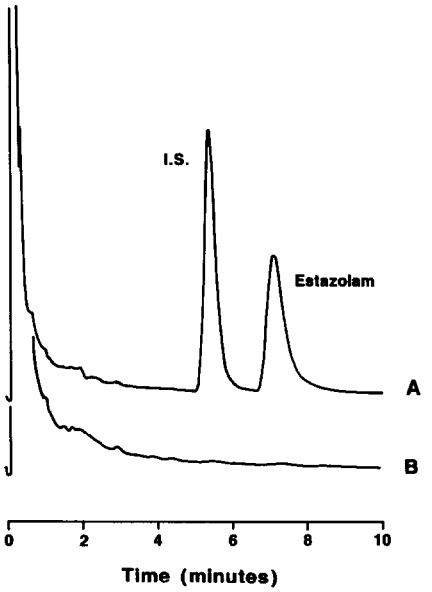

Fig. 1. A: Chromatogram of a plasma extract containing 50 ng/ml each of I.S. and estazolam. Retention times are: I.S., 5.5 min; estazolam, 7.0 min. B: Chromatogram of a drug-free control plasma extract.

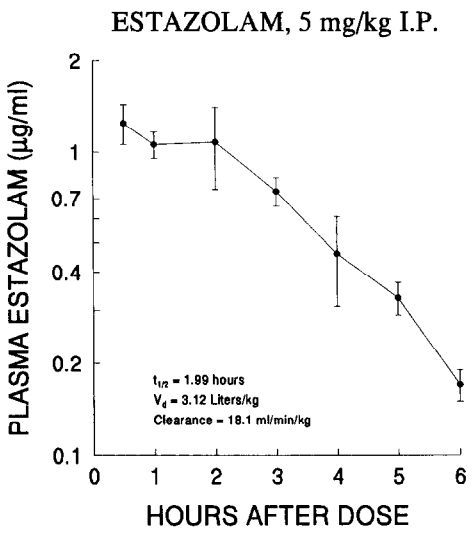

Fig. 2. Plasma estazolam concentrations following 5 mg/kg intraperitoneal injections to male CD-1 mice. Each point is the mean (± S.E.) value for 2–4 animals at the corresponding time. Pharmacokinetic parameters are listed.

intraperitoneal dose of estazolam. Derived pharmacokinetic parameters were: elimination half-life, 1.99 h; volume of distribution, 3.12 l/kg; clearance, 18.1 ml/min/kg.

CONCLUSION

This report describes a sensitive and specific method for the quantification of estazolam using gas chromatography with electron-capture detection. The method is simple and reliable and therefore appropriate for processing multiple samples.

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