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Note

Assay of triazolam in plasma by capillary gas chromatography

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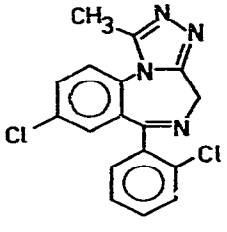
Triazolam is a triazolobenzodiazepine derivative (Fig. 1) which was recently



TRIAZOLAM

Fig. 1. Structure of triazolam.

introduced as a short-acting hypnotic. A few procedures for the determination of this drug in plasma have been described, such as radioimmunoassay [1] and high-performance liquid chromatography [2]. However, owing to a lack of sensitivity and/or specificity, these methods cannot be used to study the pharmacokinetics of this highly potent drug following administration of therapeutic doses (0.25—0.5 mg).

In this paper a gas—liquid chromatographic (GLC) method for the assay of triazolam is described that is sufficiently sensitive to be applied for pharmacokinetic studies in man. The use of a combination of solid injection, a support-coated open-tubular (SCOT) column and electron-capture detection has proved to be very suitable for this purpose.

The applicability of the method is illustrated by a preliminary study of the pharmacokinetics of triazolam in healthy volunteers.

EXPERIMENTAL

Chemicals

Triazolam as a reference substance and also triazolam tablets (0.5 mg; Halcion) were kindly supplied by the Upjohn Company (Kalamazoo, MI, U.S.A.). Clonazepam was a gift from Dr. I.C. Dijkhuis (Apotheek Haagse Ziekenhuizen, The Hague, The Netherlands). For preparing the standard solutions, ethanol (p.a. grade) (Merck, Darmstadt, G.F.R.) was used; the other organic solvents were freshly distilled (J.T. Baker, Deventer, The Netherlands).

Extraction procedure

To 2.0 ml of plasma in a centrifuge tube were added 50.0 μl of ethanol containing 5.0 ng of clonazepam (internal standard) and 0.5 ml of 0.2 *M* borate buffer (pH 9.0). After homogenization, the sample was extracted twice with 5 ml of *n*-pentane—dichloromethane (4:3) for 15 sec on a whirlmixer. After centrifugation (5 min at 2000 *g*) the upper organic layer was transferred to a silanized conical evaporation tube and evaporated to dryness at 50°C under a gentle stream of dry nitrogen. Finally, the residue was dissolved in 40 μl of ethyl acetate and 4—5 μl of this solution were brought on to a glass-lined needle of the solid injection system [3]. After evaporation of the ethyl acetate, the residue was injected into the gas chromatograph.

Apparatus and chromatographic conditions

A Hewlett-Packard Model 5713A gas chromatograph, equipped with a ^{63}Ni pulse-modified electron-capture detector and a solid injection system, was used. A SCOT column, (10 m × 0.4 mm I.D.) made of Duran 50 glass was used. The support layer was Tullanox (silanized fumed silica), particle size < 10 μm (Cabot Corp., Boston, MA, U.S.A.) and the stationary phase was 0.5% PPE-21 (Chrompack, Middelburg, The Netherlands) and 3% OV-17 (Chrompack). The operating temperatures were as follows: injection port, 350°C; column, 250°C; and detector, 350°C. The flow-rate of the carrier gas (helium) was 10 ml/min and that of the auxiliary gas (argon—methane, 95:5) was 25 ml/min.

For mass spectrometry (MS), an LKB-2091 combined gas chromatograph—mass spectrometer equipped with a PDP-11 computer system was used.

Preparation of calibration graphs

The concentration of triazolam in plasma was calculated with the aid of calibration graphs prepared by adding known amounts of triazolam to 2.0 ml of blank plasma. These standard samples were analysed by the described procedure and the ratio of the peak height of triazolam to that of the internal standard was plotted against the known concentrations of triazolam. The same procedure was followed for determining the extraction yield of triazolam from plasma at various concentrations, except that clonazepam (5.0 ng) was added after extraction as an external standard. The ratios obtained were compared with those for standard amounts of the drug.

RESULTS AND DISCUSSION

Assay procedure

Fig. 2 shows gas chromatograms of extracts of plasma samples spiked with 2.5 and 0.25 ng/ml of triazolam and with internal standard, and the gas chro-

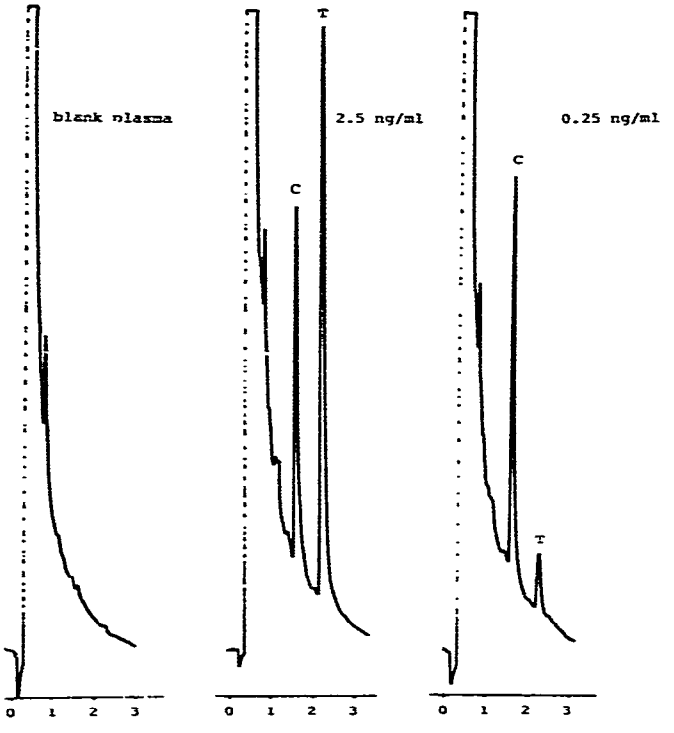


Fig. 2. Gas chromatograms obtained after extraction of 2.0 ml of blank plasma (left), 2.0 ml of plasma spiked with 2.5 ng/ml of triazolam (centre) and 2.0 ml of plasma spiked with 0.25 ng/ml of triazolam (right); the internal standard is clonazepam (C, 5.0 ng).

matogram of a blank extract. From the blank it appears that no interfering substances are co-extracted and that the retention times are short (1.5 and 2.2 min for the internal standard and triazolam, respectively). Clonazepam appears to be suitable as an internal standard, because the two peaks are well separated.

The mass spectrum of triazolam obtained from the analysis of the GLC peak was identical with the direct inlet mass spectrum (Fig. 3; mass spectrum of triazolam). In a previous study by De Boer et al. [3], it was shown that clonazepam also leaves the column unchanged. Thus both compounds appear to be determined in intact form. According to the standard curve (Fig. 4) there is good linearity between the detector response (peak height of triazolam/peak height of clonazepam) and the concentration of triazolam in the range 0.1—25 ng/ml. The correlation coefficient of such curves was not less than 0.999. Extraction yields determined in the same concentration range appeared to be constant and linear with concentration, with a mean value of 80% (the relative standard deviation at each concentration was 10% or less for $n = 3$, except for a

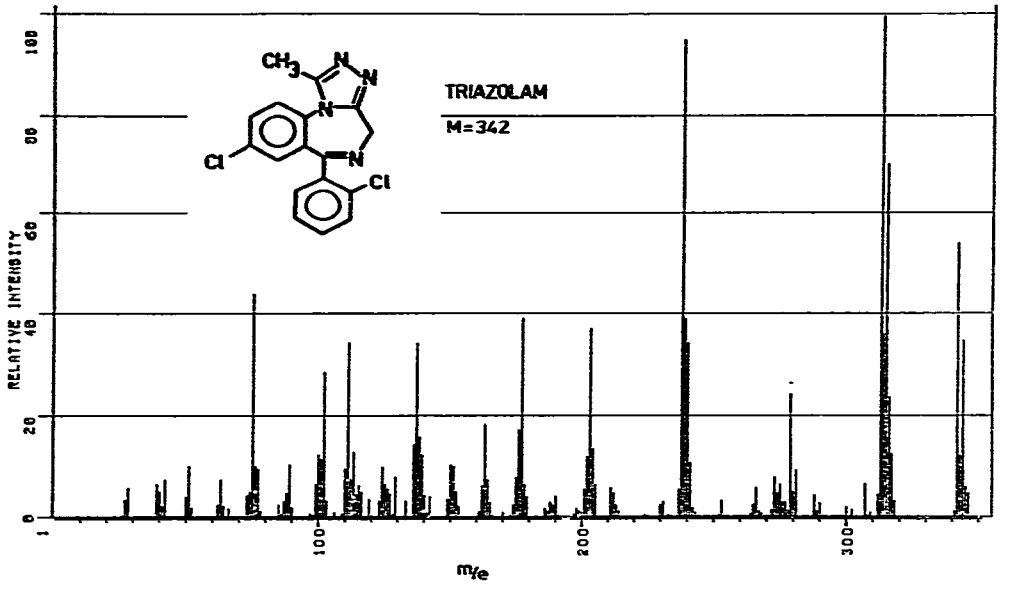


Fig. 3. Mass spectrum of triazolam obtained with the direct inlet system.

concentration of 0.1 ng/ml, where the relative standard deviation was 16.5%). Fig. 5 shows the plasma calibration graph, consisting of five individual calibration graphs for eight concentrations each. In the concentration range 0.5—25 ng/ml the relative standard deviations were below 8.5% (n = 5). The relative standard deviations for concentrations of 0.25 and 0.1 ng/ml were 17% and 16%, respectively. The correlation coefficient for each individual curve was greater than 0.999, illustrating the good linearity of the method. The lowest measurable concentration is about 0.05 ng/ml in plasma when 2.0-ml samples are used.

Human experiments

The utility of the present method for the assay of triazolam in pharmacokinetic studies in man was demonstrated by a preliminary study with healthy volunteers. Four healthy male volunteers swallowed a 0.5-mg tablet with 150 ml of tap water after an overnight fast. No food, fluid or tobacco was allowed for 3 h after drug administration. Blood samples were taken after about 20, 40, 60, 80 and 100 min and 2, 3, 4, 6, 9 and 12 h, from a forearm vein by means of a flexible venous cannula for the first 4 h and subsequently by venous puncture. Blood clotting was prevented by adding a small drop of heparin solution (5000 I.U./ml) to the samples. After separation, the plasma samples were stored at —20°C until taken for analysis, which was performed according to the procedure described above. The plasma concentration profile obtained for one volunteer is shown in Fig. 6.

Triazolam appeared to be absorbed rapidly from the tablet formulation and the elimination rate was also rapid, as shown by the short elimination half-life of about 2 h. The results are given in Table I.

The values of t_{max} (peak time), c_{max} (maximum concentration) and $t_{½}$ (elimination half-life) were similar to those found by Eberts et al. [4].

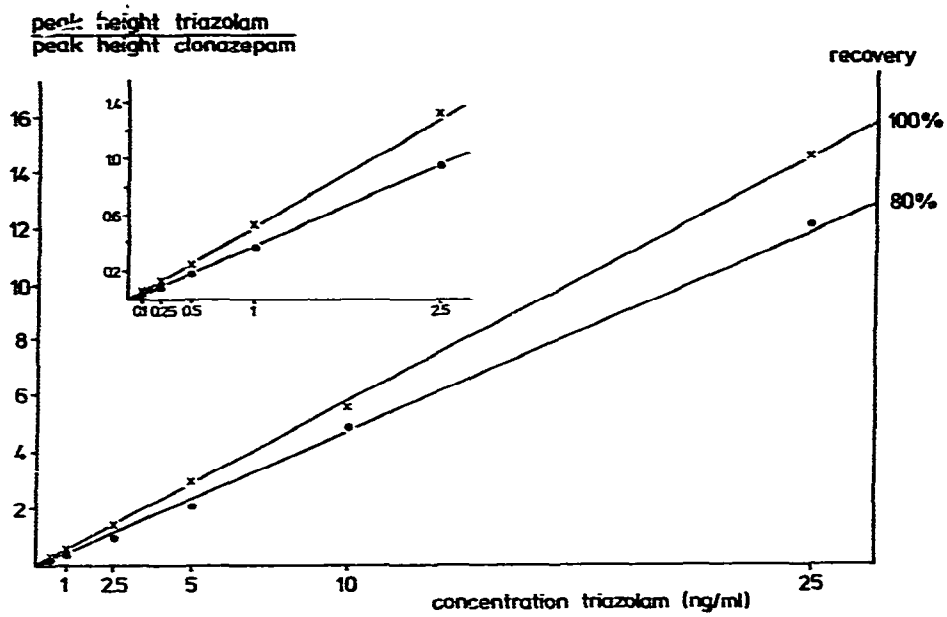


Fig. 4. Standard curve for triazolam and the calibration graph obtained after extraction from plasma using clonazepam as external standard (determination of recovery). The inset indicates on an expanded scale the recovery at low concentrations of triazolam.

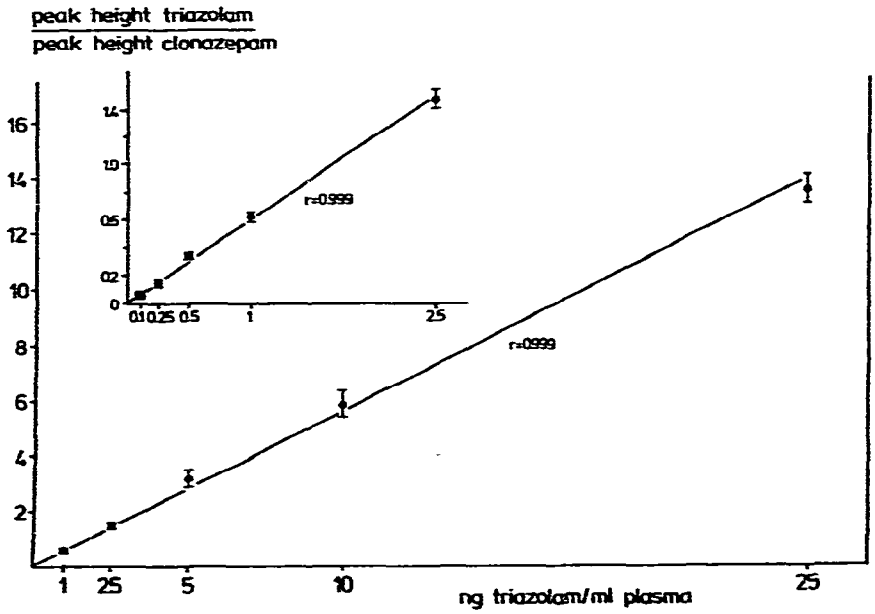


Fig. 5. Calibration graph for triazolam in plasma in the concentration range 0.1—25 ng/ml. Each point represents the mean ± S.D. of five observations. The inset indicates on an expanded scale the relationship between detector response and triazolam concentration at low concentrations of triazolam.

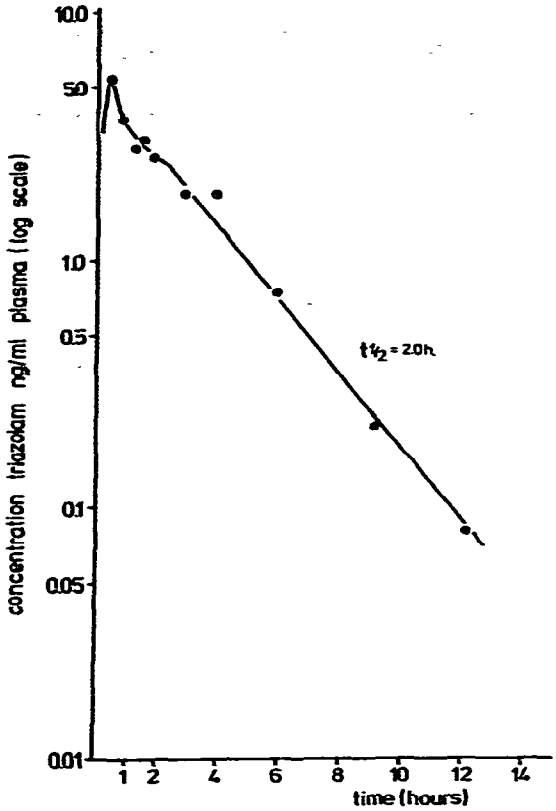


Fig. 6. Plasma concentration curve on a semi-logarithmic scale for triazolam in a healthy volunteer (F.S.) following administration of a 0.5-mg tablet (Halcion).

TABLE I

PHARMACOKINETIC PARAMETERS OF TRIAZOLAM FOLLOWING THE ADMINISTRATION OF A 0.5-mg TABLET (HALCION)

Subject	t_{max} (h)	c_{max} (ng/ml)	$t_{½\,el}$ (h)
R.G.	2	4.3	2.1
A.G.J.	1	4.4	2.3
A.N.	0.8	7.0	1.8
L.K.	1	2.5	2.0
Mean ± S.D.	1.2 ± 0.5	4.3 ± 1.9	2.1 ± 0.2

CONCLUSIONS

The procedure described for the quantitative determination of triazolam in plasma is rapid and precise. Further, with a lowest measurable concentration of about 0.05 ng/ml in plasma, it is sensitive enough for measuring the plasma concentration for at least three times the elimination half-life after therapeutic dosing.

From the results of the preliminary pharmacokinetic study in man it appears that triazolam is a benzodiazepine which is eliminated from the body very rapidly in comparison with many other benzodiazepines [5]. For a hypnotic, such a rapid elimination should be considered an advantage, because a limited duration of action may thereby be achieved [6].

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