Short communication

Determination of triazolam in serum by deactivated metal capillary gas chromatography with electron-capture detection

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Abstract

A method for the determination of trace amounts of triazolam in serum by deactivated metal capillary gas chromatography with electron-capture detection was established. The column used exhibits excellent thermostability in high-temperature analysis and easy handling and a long lifetime of the column and well shaped peaks on the chromatograms are obtained. With the metal capillary column, it was found to be easier to maintain suitable analytical conditions for the routine assay of triazolam than with a fused-silica column. With this method, 0.5 ng/ml of triazolam in serum can be determined. The method is useful for pharmacokinetic and therapeutic purposes.

Keywords: Triazolam

1. Introduction

Triazolam (Fig. 1) is one of the benzodiazepine drugs, which are widely used for the treatment of mental disorders. Administration of lower doses of triazolam are recommended because of its high potency [1]. This results in low concentrations of triazolam in blood [2], and a sensitive assay for the determination of triazolam in blood is therefore required for medical and pharmacokinetic purposes. Among the methods for the determination of triazolam, gas chromatography with electron-capture detection (GC–ECD) has high sensitivity [2–5], with the use of glass or fused-silica capillary columns. However, the column temperature has to be increased to as high as ca. 300°C because triazolam has a high boiling point among the benzodiazepines. This
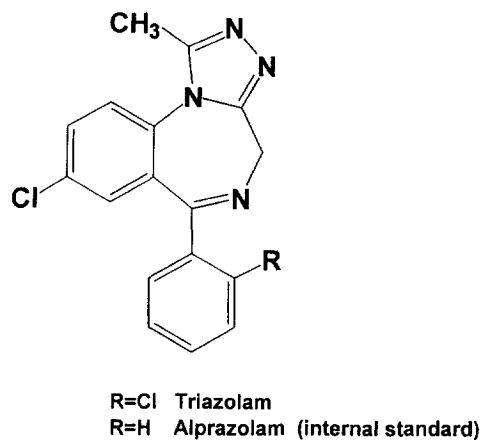

R=Cl Triazolam
R=H   Alprazolam (internal standard)

Fig. 1. Structures of triazolam and alprazoram.

causes an early deterioration of capillary columns and it is difficult to maintain suitable analytical conditions for the routine analysis of large numbers of samples.

Capillary GC started with the use of a metal capillary column, but this type of column tended to interact with the analytes and had few practical uses. Now, fused-silica capillary columns are widely used because of their inert surface and flexibility. However, in recent years, new types of deactivated metal capillary columns have been developed and their practical applications have been reported [6–10]; a RASCOT column is commercially available. There are several merits of metal capillary columns, such as mechanical toughness, flexibility and ease of handling [6], although the processes used for treatment of the inner surface are proprietary information and have not been reported. We were interested in thermostability in high-temperature GC, which is the major merit of metal capillary. We have tried to apply metal capillary column to the determination of triazolam using GC–ECD and compared it with a fused-silica capillary column.

2. Experimental

2.1. Reagents and chemicals

Drug standards of triazolam and alprazolam (internal standard) were kindly provided by Japan Upjohn (Tokyo, Japan) and Takeda Chemical Industries (Osaka, Japan), respectively. Toluene, sodium hydroxide and other chemicals were all of analytical-reagent grade from Wako (Osaka, Japan). Control serum from healthy humans was purchased from Wako.

2.2. Apparatus and analytical conditions

A GC-9APE gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a ^{63}Ni electron-capture detector was used. A RASCOT NC-17 analytical capillary column (a deactivated metal column coated with OV-17; 15 m × 0.25 mm I.D., 0.25 μm film thickness) was purchased from Nippon Chromato (Tokyo, Japan). The injector

and detector temperatures were maintained at 330°C and the column temperature programme was 110°C (held for 1 min), increased at 10°C/min to 300°C (held for 5 min). The carrier gas was helium and the make-up gas was nitrogen at a flow-rate of 40 ml/min. A Shimadzu SPL-G9 splitless injector was used for sample injection with a splitless time of 1 min. A fused-silica capillary column was used for comparative studies.

2.3. Procedure for the determination of triazolam in serum

To 1 ml of serum sample in a 15-ml centrifuge tube were added 1 ml of 0.5 M sodium hydroxide and 4 ml of toluene containing alprazolam as internal standard. The tube was shaken vigorously for 5 min and centrifuged at 1000 g for 5 min. The toluene layer was transferred into an another tube and evaporated to dryness under nitrogen at 40°C. The residue was dissolved in 0.2 ml of toluene and 3 μl were injected into the gas chromatograph.

2.4. Calibration graph

Standard samples were prepared by adding various amounts of triazolam in the range 0–10 ng/ml to serum and assayed according to the procedure described above. A calibration graph was prepared by plotting the ratio of the peak height of triazolam to that of the internal standard against concentration.

3. Results and discussion

The fused-silica column is very flexible and easy to handle in comparison with the glass column, but after use in high-temperature analysis, the material becomes brittle, which often results in breakage of the column during operation. Furthermore, several problems often occurred in assaying many routine samples using a fused-silica column. For example, the peaks of the sample showed tailing and the baseline of the chromatogram became unstable. Fig. 2 shows the
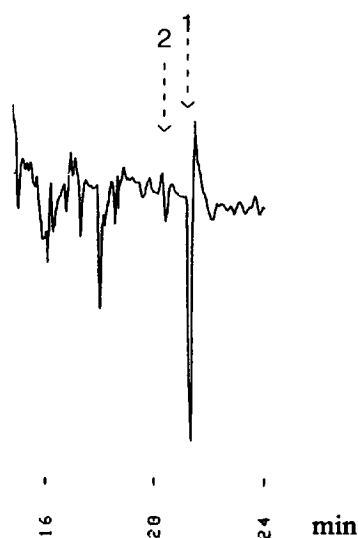
Fig. 2. Chromatogram of blank plasma using a fused-silica capillary column. Peaks: 1 = triazolam; 2 = alprazolam (internal standard).

chromatograms of blank serum obtained with the fused-silica capillary column after analyses of about 200 samples. Irregular valleys which disturb accurate determinations frequently appeared on the chromatogram. It seems that these problems are due to the deterioration of the

fused-silica capillary column in high-temperature GC.

In contrast, the chromatograms of Fig. 3 were obtained with the deactivated metal capillary column. The peaks of triazolam and internal standard do not show tailing or leading and the baseline is relatively stable. It seems that the use of metal capillary column overcomes the problem mentioned above.

Toluene was a suitable solvent because no interfering peaks were observed in the neighbourhood of triazolam and the internal standard. The limit of determination was 0.5 ng/ml in serum.

Table 1 shows the accuracy and reproducibility for triazolam; they are within acceptable precision limits for the determination of drugs in body fluids by GC–ECD, and the recovery of triazolam was almost 100%. In this method, alprazolam was used as an internal standard; when it is necessary to determine alprazolam, triazolam can be used as the internal standard without any change to the procedure. The calibration graphs for triazolam and alprazolam passed through the origin and the correlation coefficients were >0.9996 and >0.9997, respec-
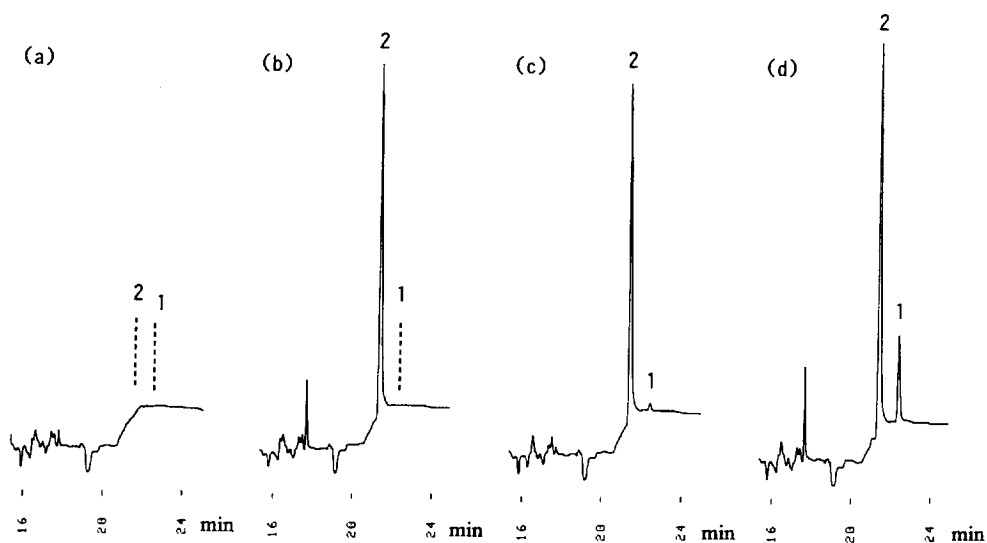

Fig. 3. Typical chromatograms of triazolam in serum, obtained using the deactivated metal capillary column. (a) Drug-free serum; (b) drug-free serum with internal standard; (c) serum with drug added (ca. 0.5 ng/ml); (d) serum with drug added (ca. 5 ng/ml). Peaks: 1 = triazolam; 2 = alprazplam (internal standard). The ordinate is the ECD response and is the same scale as in Fig. 2.

Table 1
Accuracy and reproducibility of the determination of triazolam in serum

Amount added (ng/ml)	Mean amount found (ng/ml)[a]	Relative standard deviation (%)	Relative error (%)
0.5	0.51	6.0	102.0
1.0	0.95	4.3	95.0
5.0	5.10	2.0	102.0
10.0	9.75	2.2	97.5

[a] Five replicate determinations.

tively, using the method of least squares. Hence the method developed in this study is very rapid and simple and applicable to pharmacokinetic studies or therapeutic drug monitoring of triazolam.

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