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Short communication

Determination of triazolam in a drug tablet by thermal desorption gas chromatography

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Abstract

Thermal desorption gas chromatography using a vertical microfurnace pyrolyzer was applied to determine the amount of triazolam in a tablet sample. About 1 mg of ground tablet was directly subjected to analysis without any preconcentration. When the sample was heated at 300°C, triazolam desorbed from the powder was observed as a completely isolated peak in a chromatogram together with some additional peaks formed from the main components of the tablet through pyrolysis and/or thermal desorption. The correlation coefficient of the calibration curve obtained by use of pure triazolam ranging from 2.4 to 10.5 μg was 0.999. Triazolam in the tablet sample (about 3 μg/mg) could be determined using the calibration curve. The relative standard deviation of this method was 2.4% for four runs.

Keywords: Triazolam

1. Introduction

Benzodiazepine drugs which have hypnotic and tranquilizing properties are frequently encountered in forensic casework in drug abuse and drug overdoses. Triazolam, [8-chloro-6-(*o*-chlorophenyl)-1-methyl-4H-*S*-triazolo-[4,3-*a*]-1,4-benzodiazepine] is one of the most common benzodiazepine drugs. It has recently been introduced as a short acting hypnotic drug, and is sometimes abused instead of methamphetamine and marijuana in Japan. Triazolam in biological fluids has been analyzed by high-performance liquid chromatography (HPLC) [1–4], gas chromatography (GC) [1] equipped with electron capture detector [5], gas chromatography–mass spectrometry (GC–MS) [6,7] and highly sensitive immunoassay [8]. However, HPLC, GC and GC–MS require the extraction of triazolam from biological and solid samples, while immunoassay requires expensive reagents and special apparatus. Off-line extraction methods such as solid-phase extraction [2,3] and solvent extraction [3–6] are often subjected to sample loss and/or contamination from glassware, solvents and the laboratory atmosphere, especially for trace level analysis. Furthermore, the extraction processes are not only laborious but also time-consuming.

In addition, a commercially available tablet weighing about 100 mg usually contains only a few tenths of 1 mg of triazolam. It was not an easy task to

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determine triazolam by GC after off-line extraction from one tablet. Recently, thermal desorption GC [9] has been applied to the analysis of volatile components in environmental samples [11,12] and in polymeric samples [10,11,13]. Generally, the volatile components released from the samples by thermal desorption are adsorbed on sorbents in a trap column which was placed before the separation column for GC. Then the adsorbed components are rapidly desorbed from the sorbents by heating, and introduced into the separation column. This technique has various advantages such as high sensitivity, being contamination free and rapidity.

In this article the thermal desorption technique was applied to determine triazolam in tablet samples. The ground tablet sample weighing about 1 mg was heated in a vertical microfurnace-type pyrolyzer at 300°C to release triazolam which was directly subjected to the gas chromatographic analysis. This technique proved to be a very simple, rapid, sensitive and reproducible method to determine triazolam in tablet samples.

2. Experimental

A vertical microfurnace pyrolyzer(Yanagimoto GP-1018) [14], was directly attached to a gas chromatograph (Shimadzu GC-7A) with a flame ionization detector (FID). A glass open tubular column G-100 (10 m×1.2 mm I.D.×1 μm thick coated with methyl silicone) purchased from the Chemicals Inspection and Testing Institute (Tokyo, Japan) was used. Helium was used as carrier gas (flow-rate, 25 ml min^{-1}). The temperature of the microfurnace was maintained at 300°C for thermal desorption. The temperature of the separation column was programed from 50°C to 280°C at a rate of 4°C min^{-1}. The peak identification of the chromatograms was carried out using a directly coupled GC–MS (JEOL Automass system II) to which a furnace pyrolyzer (Frontier Lab. PY-2010D) was attached.

Tablet samples named Halcion®, supplied by Upjohn (Klamazoo, MI, USA,) and reagent grade triazolam were kindly supplied by Mr. Sano (Criminal Science Laboratory, Aichi Pref. Police HQs., Japan). One tablet sample weighing about 0.1 g was ground into fine powder in a mortar before measurement. An aliquot of the sample powder weighing approximately 1 mg was put into a platinum sample cup and subjected to the thermal desorption GC measurements. Each sample taken in the sample cup was accurately weighed by a Mettler model UM-3 micro balance.

GC–MS (JEOL DX-300) using selected ion monitoring was also utilized to determine the triazolam in the tablet samples. About 0.1 g of the ground sample powder was dissolved in ethanol and filtered. The ethanol filtrate was transferred into a 10 ml volumetric flask and diluted to the mark with ethanol. Five μl of the solution was injected into the GC–MS. Packed glass column (1.5% OV-17 on Chromosorb WAW DMCS 80/100 mesh, 2 m×2.5 mm I.D.) was used to separate the triazolam, and the temperatures of the column oven, injection port and separator unit were maintained at 270, 280 and 280°C, respectively. Electron impact ionization was used at 70 eV and 300 μA.

3. Results and discussion

3.1. Identification of peaks on a chromatogram

Fig. 1 shows a typical chromatogram for the powdered Halcion sample (about 1 mg) obtained by thermal desorption GC. After the appearance of many strong peaks by 45 min, which are formed

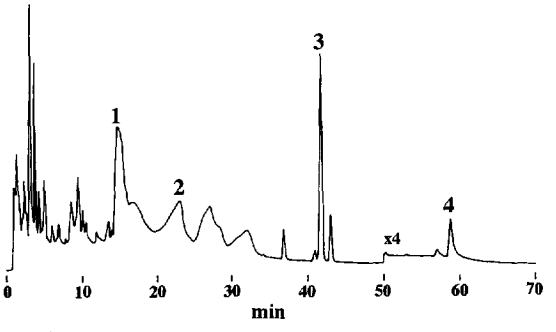

Fig. 1. Typical chromatogram of Halcion. Thermal desorption temperature was 300°C. Sample amount used was about 1 mg. Peaks are identified as follows:1=5-hydroxymethy-2-furan-carboxaldehyde; 2=levoglucosan; 3=octadecanoic acid; 4=triazolam.

through pyrolysis and/or thermal desorption of the main components, a clearly isolated peak (peak 4) of triazolam was observed at about 59 min. Major fragment ions of peak 4 were almost compatible with those of the reference spectrum for triazolam. Fragment ions at m/z 342 and 313 correspond to $[M]^+$ and $[M\text{-}2H\text{-}HCN]^+$ [5], respectively. The other large peaks shown in Fig. 1 were also identified as 5-hydroxymethyl-2-furan-carboxaldehyde (peak 1), levoglucosan (peak 2) and octadecanoic acid (peak 3), respectively. 5-Hydroxymethyl-2-furan-carboxaldehyde and levoglucosan can be attributed to the pyrolysis products of the tablet main matrix components such as starch and lactose used as excipients, diluents, bases, binders and disintegrators. Octadecanoic acid was a lubricant.

3.2. Effect of thermal desorption temperature for triazolam

Fig. 2 shows the effect of the temperature of the furnace on the peak area of triazolam desorbing form the tablet powder. The peak area of triazolam increased rapidly up to 300°C and then gently increased up to 500°C. The rapid increase up to 300°C may be attributed to the melting point of triazolam at about 240°C. Above 250°C, however, the peak areas of 5-hydroxymethyl-2-furan-carboxaldehyde, and levoglucosan drastically increased

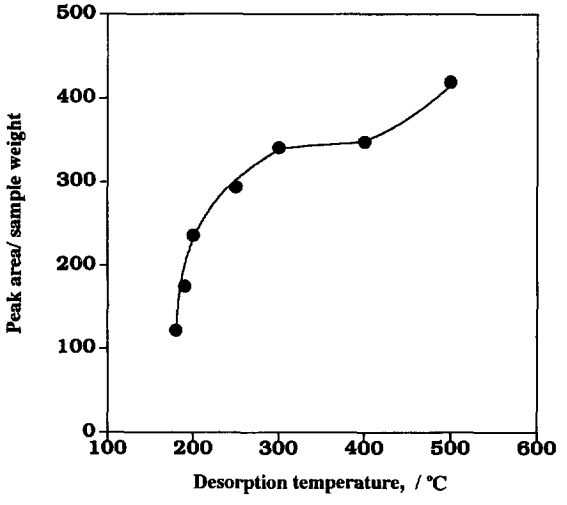

Fig. 2. Effect of microfurnace temperature on thermal desorption of triazolam.

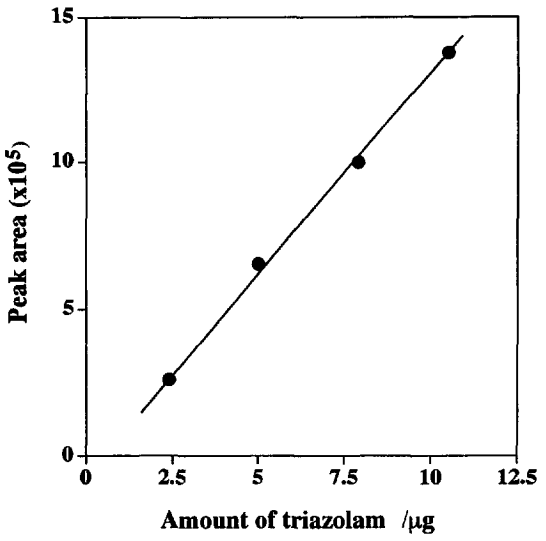

Fig. 3. Relationship between amount of triazolam and observed peak area.

with temperature. This means that the matrix compounds of the tablet, such as starch and lactose, were largely pyrolyzed above 250°C. Almost constant recovery of triazolam from the tablet matrix was obtained between 300 and 400°C. However, above 400°C, tailing peak of the pyrolysates from the matrix components became significant to affect the peak of triazolam.

On the other hand, when neat triazolam sample (about 10 μg) is desorbed in the furnace at 300°C, only a single peak of triazolam was observed at about 59 min without any accompanying degradation peaks. Furthermore, the same tendency was observed for triazolam in the presence of the approximately 1 mg of lactose at 300°C, except for additional octadecanoic acid. These facts suggest that triazolam in the tablet matrix is almost quantitatively recovered at the desorption temperature of 300°C without causing any appreciable degradation. Thus, in the following the thermal desorption of triazolam was carried out at 300°C.

3.3. Determination of triazolam in drug tablet

In order to evaluate the linearity of the calibration curve, known amounts of pure triazolam ranging from 2.4 to 10.5 μg which were weighed into the

Table 1
Reproducibility of peak area of triazolam

Sample weight (μg)	Observed peak area	Peak area/sample weight (μg^{-1})
1109.1	416 246	375.3
1148.0	413 618	360.3
1130.5	409 835	362.5
1034.8	368 826	356.4

platinum sample cup were subjected to thermal desorption GC at 300°C. Fig. 3 shows the observed calibration curve. The correlation coefficient of the curve was 0.999. Moreover, the peak areas of the triazolam from control samples, where triazolam was coexistent with about 1 mg of lactose, were on the same calibration curve.

Triazolam in two different tablet samples was determined using the calibration curve. The observed triazolam contents were 2.9 μg/mg in both the tablet samples. The relative standard deviation for the relative peak area of triazolam was 2.3% for four runs using about 1 mg of the powdered sample obtained by one tablet as shown in Table 1. Since the manufacturer specifies that one tablet sample weighing 96–97 mg contains 0.25 mg of triazolam, its content in the tablet is to be 2.6 μg/mg. Moreover, the reference triazolam contents determined by the GC–MS method were between 2.7 and 2.8 μg/mg for the three different tablet samples. These data suggest that fairly accurate determination of triazolam is obtained by the proposed thermal desorption GC without using any preliminary sample treatment.

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