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Determination of benzodiazepine in tablets studied by thermal desorption gas chromatography

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Abstract

Thermal desorption gas chromatography (TDGC) was applied to the analysis of 13 kinds of tablets containing different benzodiazepines (BZDs). An aliquot of ground tablet sample (0.1–1 mg), weighed into a platinum sample cup, was placed in a furnace pyrolyzer where the sample was heated up to a suitable temperature (150–500°C) so that BZD was desorbed from the sample powder. The desorbed components of BZDs were immediately transferred into a separation column by a helium carrier gas without using any trapping techniques. The desorbed components were identified by TDGC-mass spectrometry. Among various BZDs, fludiazepam, nimetazepam and mexazolam in tablet samples were determined by the present method. Thus, the results obtained were in good agreement with the specified values. Correlation coefficients of the calibration lines for the three BZDs' ranged from 0.997 to 0.999 for several micrograms to about 10 μg of the components. Relative standard deviations of this method were $< 4.1\%$ for 4 or 5 runs.

Keywords: Benzodiazepines; Thermal desorption; Thermal desorption gas chromatography; Fludiazepam; Nimetazepam; Mexazolam

1. Introduction

Benzodiazepines (BZDs) are frequently used as tranquilizers, sleep inducers, anti-epileptics and muscle relaxants. Unfortunately, these drugs are also subject to abuse, tolerance and dependence [1]. Therefore, BZDs are often encountered in forensic caseworks involving drug abuse, road traffic offences and/or drug overdoses. Many chromatographic methods have been applied to the analysis of the BZDs and their metabolites [2–6]. Immunological assays [5,7,8] were used with the advantage of determining and/or screening the excretion of urinary BZDs and their metabolites. Polarographic methods have been also applied for the determination of several BZDs in urine [9].

From the point of view of forensic scientists, there are three important requirements for the analysis of BZDs. Firstly, not only biological

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Table 1
List of benzodiazepine tablet samples

No.	Trade name of tablet	BZD compound	Content per tablet (mg)[a]	Weight of tablet (mg)	Weight (%)[b]
1	Eurodin	Estazolam	2	121.3	1.65
2	Sepazon	Cloxazolam	2	119.6	1.67
3	Cercine	Ddiazepam	2	90.7	2.21
4	Halcion	Triazolam	0.25	97.4	0.257
5	Nelbon	Nitrazepam	5	453.7	1.10
6	Erimin	Nimetazepam	3	170.6	1.76
7	Erispan	Fludiazepam	0.25	80.1	0.312
8	Silece	Flunitrazepam	1	101.0	0.990
9	Lendormin	Brotizolam	0.25	149.8	0.167
10	Melex	Mexazolam	0.5	81.0	0.617
11	Resmit	Medazepam	5	119.4	4.19
12	Wypax	Lorazepam	0.5	101.1	0.495
13	Balance	Chlordiazepoxide	10	153.8	6.50

[a] Manufactures' specified values.
[b] Specified values.

fluids samples but also tablets should be subject to analysis; secondly, the method should be sensitive enough for only a small amount of sample; and finally, the method should be accurate and rapid enough for the actual needs. In addition, sample preparation should be as simple as possible because pretreatment processes may cause sample loss as well as contamination from glassware, solvents and the laboratory atmosphere. In particular, conventional solvent extraction processes from the solid samples which are often tedious and time-consuming should be avoided if possible.

In addition, since the content of BZDs in tablets available from many manufacturers is $<$ 1% for each BZD component, it is often difficult to analyze by gas chromatography (GC) after the off-line extraction using the mg range of the tablet sample. On the other hand, thermal desorption gas chromatography (TDGC) is a well-established analytical technique which has been applied in the direct determination of volatile compounds in solid samples [10,11]. Recently, we have shown that TDGC is effective for the rapid determination of triazolam in about 1 mg of a tablet sample containing about 0.3% triazolam in a large amount of matrix compounds such as lactose and octadecanoic acid [12]. In this paper, three kinds of BZDs such as fludiazepam, nimetazepam and mexazolam were determined in about 1 mg of the tablet samples by means of TDGC.

2. Experimental

2.1. Materials

In Table 1, 13 kinds of tablet samples are listed together with their main BZD components and their contents. In addition, reagent grade BZDs were also used. These tablet samples were kindly supplied by a pharmacist in Meijo Hospital, while the neat BZD samples were provided by Mr Sano in our laboratory. Their structural formulae are shown in Fig. 1. The tablet and the neat samples were ground into a fine powder in a mortar before measurement. An aliquot of the tablet sample powder (0.1–1 mg) or the neat sample powder (about 2–12 μg) was placed in a platinum cup and subjected to the TDGC measurements.

2.2. Apparatus

The TDGC, TDGC-mass spectrometry (MS) systems and micro balance with submicrogram sensitivity utilized and their operating conditions were basically the same as described in our previous paper [12]. Simultaneous thermogravimetric

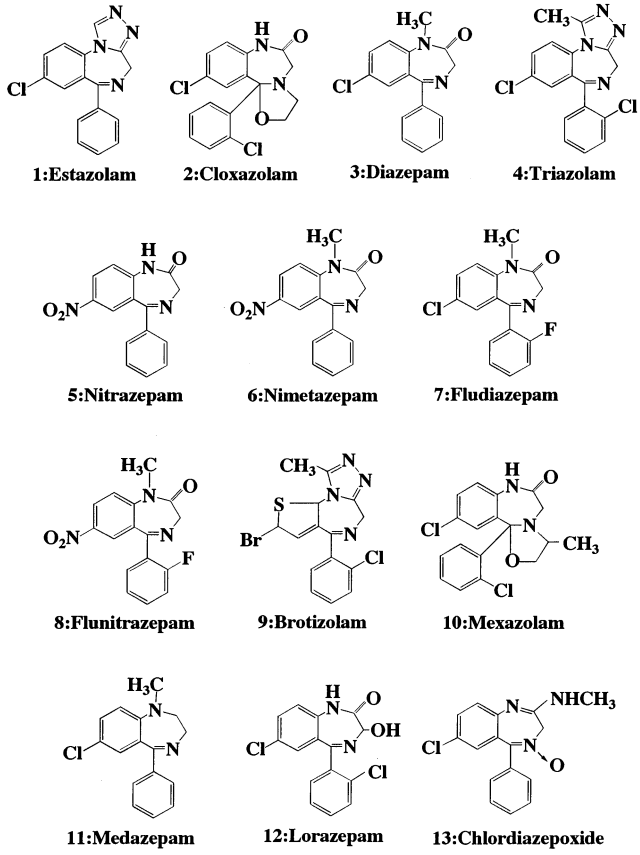

Fig. 1. Structural formulas of benzodiazepines used.

(TG) and differential thermal analysis for the tablet and the neat samples was performed using a Rigaku Thermal Analysis System 300 interfaced with a computerized data processing system.

3. Results and discussion

3.1. Effect of temperature on thermal desorption of BZDs

Fig. 2 shows the chromatograms of fludiazepam desorbed from Erispan® obtained at various desorption temperatures between 120 and 200°C. A clearly isolated peak (peak 3), which was identified as fludiazepam from the mass spectrum, was observed at about 45 min. The peak was not interfered with from any pyrolysis products of the matrix components at any temperature. The peak area of fludiazepam increased drastically up to 170°C and decreased gradually above 170°C, mainly due to the accompanied decomposition of fludiazepam during its thermal desorption. Below 170°C, fludiazepam proved to be desorbed from the tablet sample without causing any pyrolysis of the matrix components because only one peak of the component was observed on the chromatogram. When the thermal desorption temperature was set at 200°C, several peaks were additionally observed before the peak of fludiazepam. Two of these peaks were identified as 5-hydroxymethyl-2-furan-carboxaldehyde (peak 1) and levoglucosan (peak 2). These compounds are attributed to the tablet main components such as lactose

Table 2
Analytical results of neat benzodiazepines

No.	Compound	Number of observed peaks by TDGC	Melting point (°C)[a]	Decomposition temperature (°C)[a]
1	Estazolam	1	230.7	339.9
2	Cloxazolam	12	214.0	339.9
3	Diazepam	1	132.3	282.9
4	Triazolam	1	241.1	344.5
5	Nitrazepam	3	227.8	285.4
6	Nimetazepam	3	161.6	253.4
7	Fludiazepam	1	95.5	–
8	Flunitrazepam	3	169.8	268.4
9	Brotizolam	2	210.4	299.0
10	Mexazolam	10	194.5	223.0
11	Medazepam	1	102.0	248.7
12	Lorazepam	1	181.5	273.5
13	Chlordiazepoxide	7	242.7	284.2

[a] Obtained from TA.

and starch used as excipients, diluents bases and binders [12]. In addition, octadecanoic acid which was used as a lubricant, was also observed in the chromatograms of some other tablet samples.

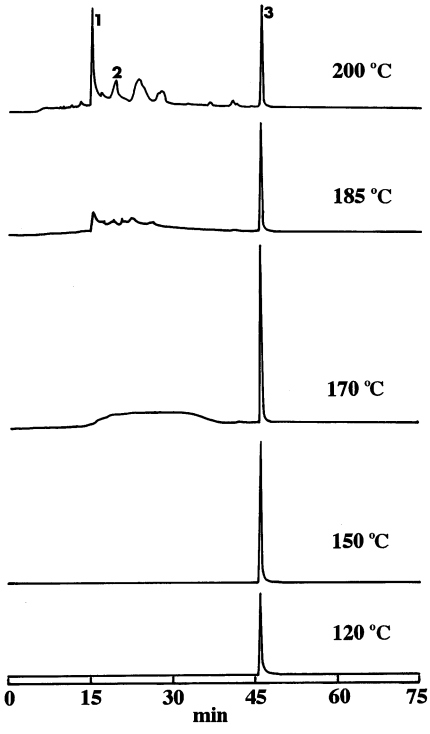

Fig. 2. Effect of temperature on desorption of fludiazepam from Erispan®. Approximately 1 mg of Erispan® was used: (1) 5-hydroxymethyl-2-furan-carboxaldehyde; (2) levoglucosan; and (3) fludiazepam.

The number of observed peaks in the chromatograms of the neat BZDs is summarized in Table 2, together with their melting points and decomposition temperatures measured by TA. Among these, the peak of BZD itself was identified in each chromatogram by TDGC-MS, with the exception of nitrazepam. Some of the BZDs yielded only one peak arising from the corresponding molecule while the others yielded several additional peaks associated with the pyrolysis products of the corresponding BZDs. The former type comprised estazolam (No. 1), diazepam (No. 3), triazolam (No. 4), fludiazepam (No. 7), medazepam (No. 11) and lorazepam (No. 12). The other BZDs belong to the latter class. It is interesting to note that the melting points of the former type are generally much lower than their decomposition temperatures, while those of the latter type are almost comparable. In particular, the four samples such as cloxazolam (No. 2), nitrazepam (No. 5), mexazolam (No. 10) and chlordiazepoxide (No. 13), of which melting and decomposition temperatures are very close to each other, yielded three to twelve peaks, suggesting their thermal instability. For the following examination, the observed molecular peaks were mostly used. However, as for nitrazepam and mexazo-

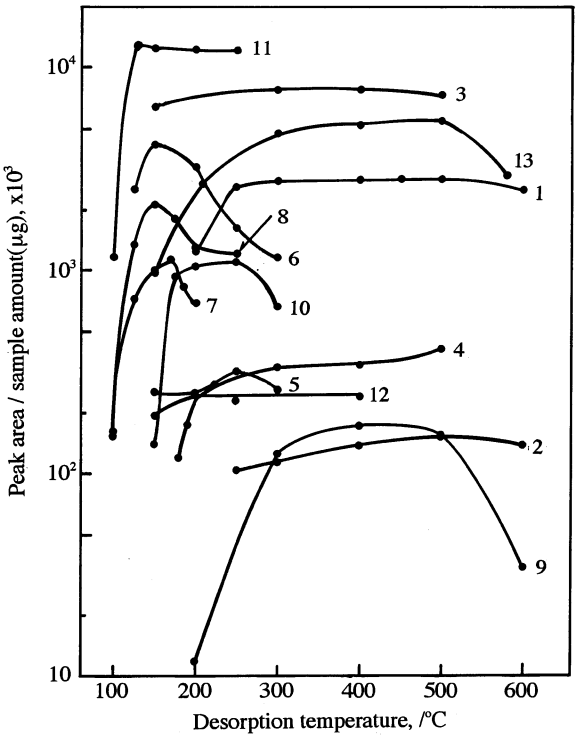

Fig. 3. Effect of the desorption temperature on peak areas of benzodiazepines for tablet samples. The numbers correspond to those in Table 1.

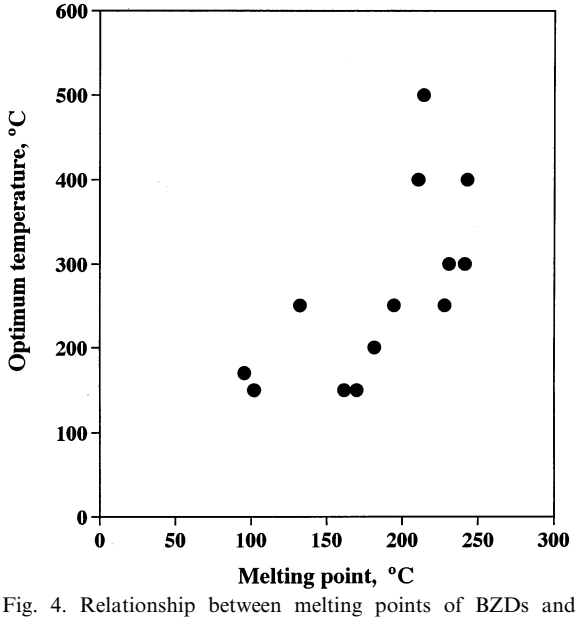

Fig. 4. Relationship between melting points of BZDs and optimum temperature of thermal desorption from tablet.

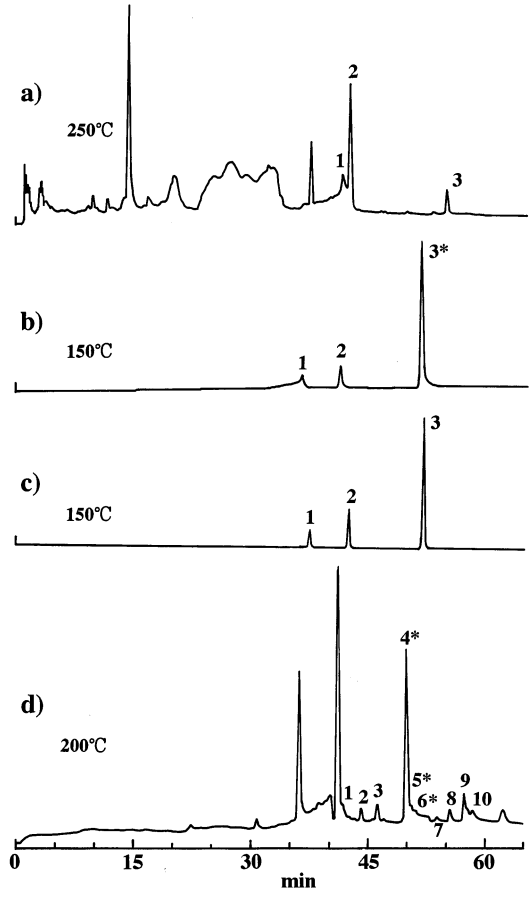

Fig. 5. Chromatograms of Nelbon®, Erimin®, Silece® and Melex®. Asterisked peaks were used in determining nimetazepam and mexazolam, respectively: (a) Nelbon®; (b) Erimin®; (c) Silece®; and (d) Melex®.

lam, the strongest peaks (unidentified) among the observed peaks were used instead.

Fig. 3 illustrates the effects of the thermal desorption temperature on the peak areas of the thirteen BZDs from the tablet samples containing the corresponding components. Although the peak areas of the observed molecular BZDs for Erimin® (No. 6), Erispan® (No. 7), Silece® (No. 8) and Melex® (No. 10) and that of the strongest peak for Nelbon® (No. 5) changed drastically throughout the entire desorption temperature tested, while those of the other samples had plateau regions where the peak areas became almost constant.

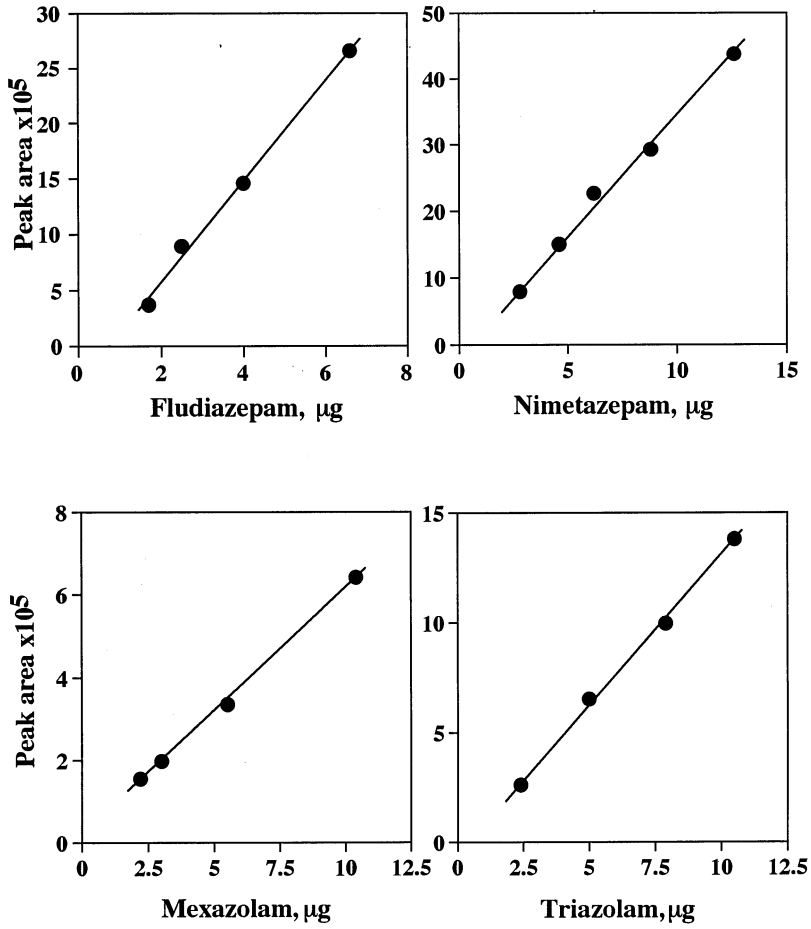

Fig. 6. Calibration curves for fludiazepam, nimetazepam, mexazolam and triazolam.

Fig. 4 shows the relationship between the optimum temperatures, which were defined as the temperatures for obtaining maximum peak areas or the starting temperatures of a plateau region, and the melting points of BZDs. As might be expected, it is interesting to note that fairly good correlation exists between them.

3.2. Determination of fludiazepam, nimetazepam and mexazolam in drug tablets

In our previous work [12], triazolam in a tablet was successfully determined by TDGC at 300°C, which showed almost constant peak intensities between 300 and 400°C. This result suggests that the BZDs indicating plateau regions in Fig. 3 such as estazolam (No. 1), diazepam (No. 3), medazepam (No. 11), etc. must be determined by TDGC in a same manner. Therefore, in this work, the BZDs of which peak intensities drastically change depending on the thermal desorption temperature, were subjected for the determination by TDGC.

Fig. 5 shows the thermal desorption chromatograms of nitrazepam, nimetazepam, flunitrazepam and mexazolam from Nelbon®, Erimin®, Silece® and Melex® respectively at optimum desorption temperature. In Fig. 5, numbered peaks in each chromatogram were also observed in the chromatograms of the corresponding neat samples. As for Nelbon® at 250°C, many peaks from the tablet matrix components

Table 3
Quantitative results for four benzodiazepines

Compound	TD temperature(°C)[a]	Specified weight (%)[b]	Measured weight (%)	RSD (%)[c]	Correlation coefficient
Fludiazepam	170	0.312	0.35	1.35 ($n=5$)	$r=0.998$
Nimetazepam	150	1.76	1.75	4.12 ($n=4$)	$r=0.997$
Mexazolam	200	0.617	0.66	2.53 ($n=4$)	$r=0.999$
Triazolam[d]	300	0.257	0.29	2.25 ($n=4$)	$r=0.999$

[a] Thermal desorption temperature.
[b] Refer to Table 1.
[c] Relative standard deviation.
[d] Reference [12].

and their pyrolysis products were also observed as well as the pyrolysis products of nitrazepam. However, the molecular peak of nitrazepam was not observed. On the other hand, when Erimin® and Silece® were heated at 150°C, nimetazepam (peak 3*) and flunitrazepam (peak 3) were desorbed from their tablet samples together with their pyrolysis products (peaks 1 and 2), but without causing any pyrolysis of the tablet matrix components. Furthermore, pyrolysis products of mexazolam and those of tablet matrix components appeared with mexazolam (peak 10) on the chromatogram of Melex® desorbed at 200°C.

In the present study, fludiazepam (No. 7), nimetazepam (No. 6) and mexazolam (No. 10) were determined by TDGC because of the following reasons. Fludiazepam (Erispan®) at 170°C was used for the example where only one peak was obtained from the tablet sample, i.e. the pyrolysis products of fludiazepam and those of the tablet matrix components were not observed on the chromatogram. Nimetazepam (Erimin®) was selected as a typical sample for which thermal degradation of the intact molecule occurred to some extent. As for mexazolam (Melex®), due to the insufficient separation of peaks, its pyrolysis products (peaks 4–6) were used for determination instead of the molecular peak (peak 10). Asterisked peaks were used for the determination of nimetazepam and mexazolam, as shown in Fig. 5. Nitrazepam and flunitrazepam were not determined in this work because the desorption species from Nelbon® (No. 5) were unidentified by TDGC-MS and chromatogram of flunitrazepam from Silece® was very similar to that of nimetazepam from Erimin®, although the peak areas of the nitrazepam (No. 5) and flunitrazepam (No. 8) also drastically changed depending on the thermal desorption temperature as shown in Fig. 3.

TDGC measurements were carried out at the optimum temperatures indicated in Fig. 3, i.e. 150, 170 and 200°C for nimetazepam (No. 6), fludiazepam (No. 7) and mexazolam (No. 10), respectively. Fig. 6 shows typical calibration lines for fludiazepam, nimetazepam, mexazolam, along with that of triazolam at 300°C. The correlation coefficients of the lines were between 0.997 and 0.999. The amount of BZDs in a given tablet was determined using these calibration lines. Thus, obtained results are summarized in Table 3 for the four BZDs including triazolam. The observed results proved to be in fair agreement with the reference values. This fact suggests that even the BZDs, of which desorbing yields are strongly affected by the desorption temperature, can also be determined by TDGC using tablet samples of <1 mg.

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