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Short Communication

Gas chromatographic determination of zopiclone in plasma after solid-phase extraction

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ABSTRACT

A gas chromatographic technique for determining zopiclone based on a solid-phase extraction procedure with C_{18} cartridge for sample clean-up is presented. Quantification can be achieved with 1 ml of plasma. The method uses prazepam as internal standard. Zopiclone is separated on a 5% phenyl methyl silicone analytical column and detected with an electron-capture detector, which consequently allows a limit of quantitation of 2 μg/l. It is thus simple, rapid, sensitive and linear over the range 5–2000 μg/l.

INTRODUCTION

Zopiclone, a cyclopyrrolone derivative, is a non-benzodiazepine hypnotic drug possessing a short duration of action and few associated side effects [1–3]. The therapeutic dose in adults is 7.5 mg of zopiclone orally, and plasma levels are in the range 20–80 μg/l [4,5].

Several methods have been reported to detect zopiclone in plasma or serum: gas chromatography (GC) [6,7] and column liquid chromatography (LC) with fluorescence detection [8–10] or more recently with ultraviolet detection [11,12]. All involve a liquid–liquid extraction of zopiclone before assaying the drug. Therefore, we have developed a solid-phase extraction (SPE) well suited for emergency cases. It is simple, sensitive and reproducible, and the range of linearity allows pharmacological or toxicological determinations.

EXPERIMENTAL

Instrumentation

For SPE, we used a Vac Elut sample-processing station (Analytichem International) from Prolabo (Paris, France) and C_{18} extraction columns, 100 mg/ml, from Alltech (Templeuve, France).

Stock solutions of zopiclone and prazepam (internal standard) and standards were prepared with an SMI 200 liquid-handling unit from Unipath (Lyon, France).

The GC workstation consisted of a Hewlett-Packard HP 5890 Series II gas chromatograph (Les Ulis, France) equipped with an electron-capture detector and a capillary on-column injection inlet system. The column was a 25 m ×

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0.32 mm I.D. column coated with Ultra 2 (5% phenyl methyl silicone, film thickness 0.52 μm) from Hewlett-Packard (Avondale, PA, USA).

The detector was maintained at 320°C. The carrier gas was helium (inlet pressure 100 kPa giving a flow-rate of 1.8 ml/min) and the make-up gas was nitrogen at a flow-rate of 60 ml/min. Signals from the detector were recorded by HP 3365 software, which also monitored the HP 5890 gas chromatograph, and an HP 7673 automatic injector.

A 1 m × 0.53 mm I.D. SE-30 fused-silica capillary column was used as a pre-column and was changed each week. The pre-column was connected to the analytical column with a glass press fit connector of 0.32–0.53 mm. Injection (1 μl) was done at 213°C (oven track mode). The oven temperature was 210°C, increased thereafter to 300°C at 2.5°C/min and held at that temperature for 9 min. The chromatography time was 40 min. Retention times were: zopiclone, 11.8 min, and prazepam, 22.7 min.

Chemicals

Zopiclone (Rhône-Poulenc Santé, Courbevoie, France) and the internal standard prazepam (Park-Davis France, Orléans, France) were gifts from the manufacturers.

Methanol, isopropanol and chloroform were HPLC grade from Prolabo. Potassium hydroxide (KOH) was of analytical grade from Merck (Nogent sur Marne, France). Acetic acid, *n*-hexane and toluene were HPLC grade from Merck. Stock solutions (1 mg/ml) of zopiclone and prazepam were prepared by dissolving 50 mg of free base in 50 ml of toluene for zopiclone and 50 ml of isopropanol for prazepam and stored at 4°C. The working standard was prepared in human plasma to contain 2 mg/l zopiclone and stored in 2-ml screw-cap glass vials at −30°C until assay. Other points of the range of calibration were obtained by diluting this standard to 1:9, 1:99 or 1:399 with drug-free plasma.

Internal standard working solution was prepared by diluting 100 μl of the isopropanolic stock solution (1 mg/ml) of prazepam in 99.9 ml of a 0.001 *M* aqueous KOH solution (pH 11). This working solution was stored in 10 ml screw-cap glass vials at 4°C until assay.

Solid phase extraction

A 1-ml aliquot of working internal standard solution was added to 1 ml of plasma standard or unknown sample, then vortex-mixed for 15 s. Extraction cartridges installed on the Vac Elut were conditioned with 2 ml of methanol followed by 1 ml of 0.001 *M* KOH. Prepared plasma samples or standards were then added to their corresponding cartridges and aspirated under vacuum. Columns were washed once with 500 μl of deionized water and once with 500 μl of *n*-hexane. The analytes were eluted with three volumes of 200 μl of 0.5% acetic acid in methanol–chloroform (9:1, v/v). The eluate was evaporated under a stream of nitrogen at 50°C. Next, just before injection on the GC column, the residue was dissolved in 50 μl of isopropanol and a 1-μl volume of this was injected.

RESULTS AND DISCUSSION

Stability of zopiclone

The first GC assay of zopiclone was performed by Kennel *et al.* [7]. They identified zopiclone as a single peak eluting slightly before diazepam. Consideration of the molecular mass of zopiclone (*i.e.* 388 compared with the diazepam molecular mass of 284) suggests that the zopiclone is not the compound described by Kennel *et al.* [7]. A recent paper by Boniface *et al.* [12] demonstrated that zopiclone may be decomposed under the thermal conditions of GC analysis to give smaller decomposition products (Fig. 1). The two major peaks were analysed by mass spectrometry by the same authors: peak 2 was identified as zopiclone, while the authors proposed a structure for the compound of peak 1 (compound V) (Fig. 2). The ratios were dependent on the injection mode (*i.e.* split or splitless), the injector port temperature and especially on the injection solvent.

According to Boniface *et al.* [12] zopiclone is very unstable in nucleophilic solvents such as methanol or ethanol. Stability seems to be slightly better in isopropanol, acetonitrile and toluene.

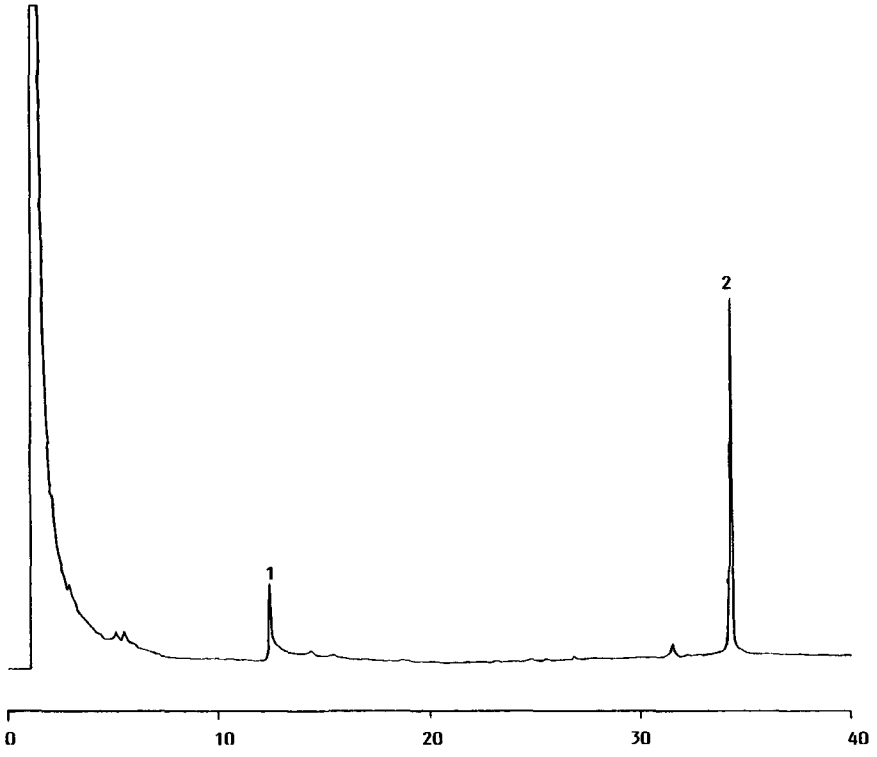

Fig. 1. GC–ECD profile of 50 ng of zopiclone standard in acetonitrile. Peaks: 1 = compound V; 2 = zopiclone.

Because of these problems plasma standards were prepared with zopiclone freshly dissolved in toluene.

Unfortunately the degradation of zopiclone to compound V is not correlated with time, and it seems that a GC assay is not valuable for zopiclone. For this reason we have developed an SPE which allows simultaneously complete transformation of zopiclone into compound V and an efficient extraction of the drug. After SPE, the chromatogram shows a single peak corresponding to compound V (Fig. 3a–c). This SPE permits a reproducible GC assay of zopiclone in plasma.

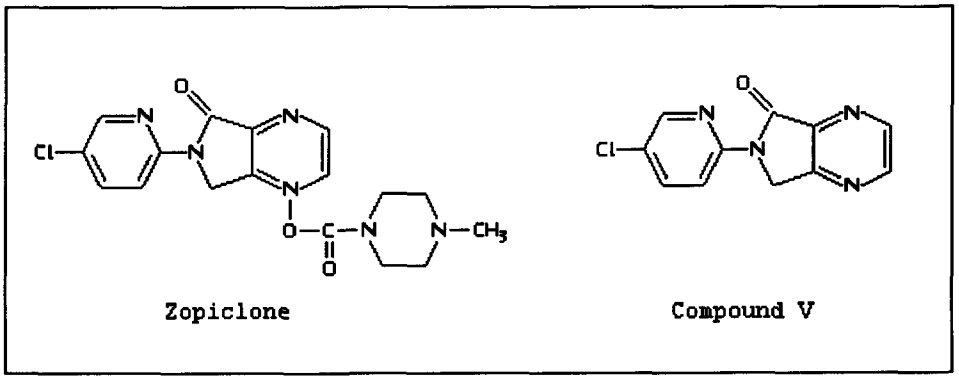

Fig. 2. Structures of zopiclone and compound V.

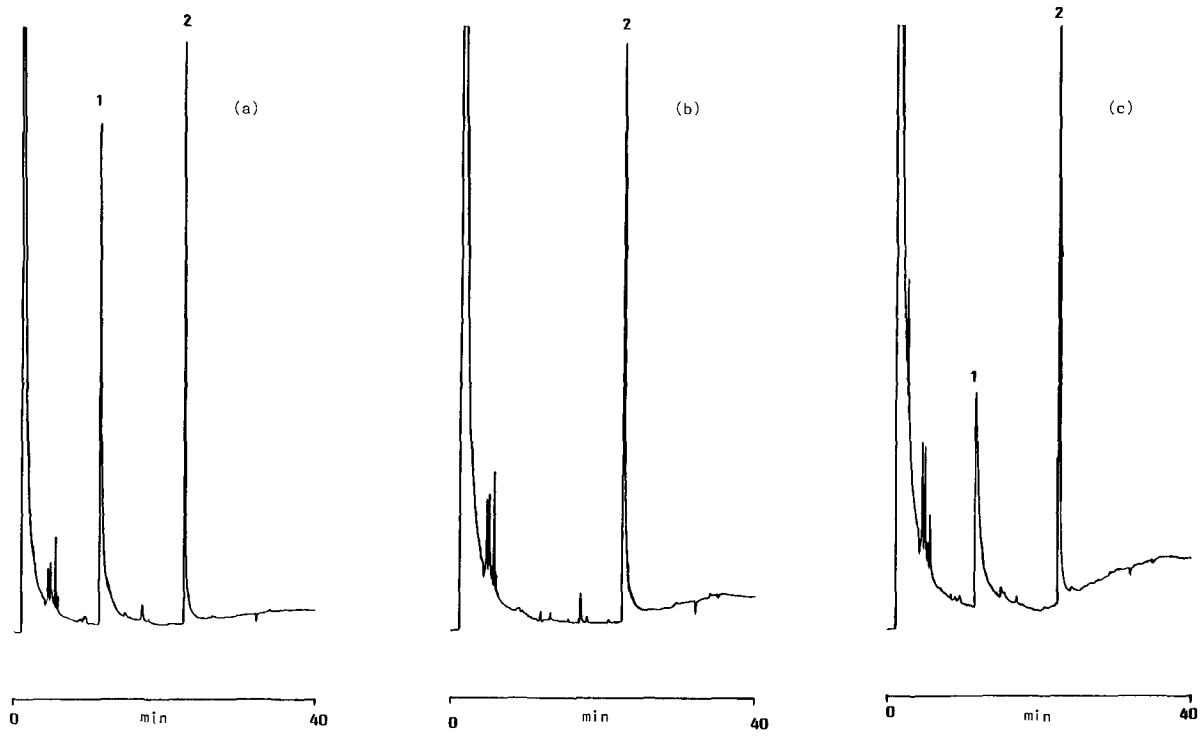

Fig. 3. Chromatograms of (a) an extract of 1 ml of plasma containing 2 μg of zopiclone, (b) an extract of blank plasma, and (c) an extract of plasma from a patient 1.5 h after a therapeutic oral dose of 7.5 mg of zopiclone (zopiclone = 63 μg/l). Peaks: 1 = compound V; 2 = internal standard.

Extraction recovery

The extraction recovery, determined by comparing peak areas of extracted plasma and methanolic standards (*i.e.* compound V) at the same concentration, is 97.4% (C.V. = 1.8%) at 2 mg/l (n = 10) and 98.0% (C.V. = 2.6%) at 200 μg/l (n = 6).

Linearity and detection limit

The calibration curve was linear over the range 5–2000 μg/l. The regression equation (n = 8) and the correlation coefficient for zopiclone are $y = 0.00076867x - 0.00128733$ and $r = 0.997$, respectively.

The within- and between-day precision are presented in Table I.

TABLE I

METHOD VALIDATION PARAMETERS

Known values (μg/l)	Within-day precision (n = 8)		Between-day precision (n = 10)		Accuracy (mean ± S.D., n = 10) (μg/l)
	Mean ± S.D.[a]	C.V. (%)	Mean ± S.D.[a]	C.V. (%)	
5	0.00365 ± 0.00026	7.1	0.00362 ± 0.00031	8.7	5.81 ± 0.51
20	0.01541 ± 0.00105	6.8	0.01524 ± 0.00158	7.6	21.90 ± 1.66
200	0.14682 ± 0.00719	4.9	0.15001 ± 0.00765	5.1	192.65 ± 9.83
2000	1.53633 ± 0.08296	5.4	1.49470 ± 0.10163	6.8	1946.18 ± 132.34

[a] Ratio of peak-area response of the analyte to peak-area response of the internal standard.

The limit of quantification was determined by spiking plasma with decreasing concentrations of zopiclone until a response equivalent to twice the background was observed. It was found to be 2 μg/l (C.V. = 9.6%, n = 8). This value is adequate for pharmacokinetic studies.

Interferences

Salicylates, barbiturates, meprobamate, digoxin, digitoxin, theophylline, acetaminophen, valproic acid and phenytoin are not detected by this method. Normal serum components or other drugs tested did not interfere with the analysis of zopiclone. Benzodiazepines, tricyclic antidepressants, carbamazepine, alpidem, zolpidem and buspirone have retention times longer than 12.5 min.

CONCLUSION

This first SPE of zopiclone provides faster, more efficient and cheaper sample preparation than was possible with traditional liquid–liquid procedures. The proposed method is rapid, linear over a wide range, sensitive, reproducible and valuable for toxicological and pharmacological studies.

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