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Short Communication

Determination of zopiclone in plasma using column liquid chromatography with ultraviolet detection

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ABSTRACT

A reversed-phase liquid chromatographic method with ultraviolet detection for the determination of zopiclone in plasma is described. It is rapid, sensitive, reproducible and linear over a wide range. The method was used to study plasma zopiclone concentrations in a case of acute intoxication after oral ingestion of 300 mg of the drug. The plasma level was 1600 ng/ml 4.5 h after the dose and the elimination half-life was 3.5 h.

INTRODUCTION

Zopiclone (Imovane; Theraplix, Rhône Poulenc, Paris, France) [1], a cyclopyrrolone derivative, is a sedative hypnotic agent possessing a short duration of action. The maximum plasma level is about 80 ng/ml with a therapeutically active hypnotic dose of 7.5 mg [2]. Gas chromatographic [3,4] and column liquid chromatographic (LC) with fluorescence detection [5–9] methods have been reported for the determination of zopiclone.

This paper describes an assay method for zopiclone in plasma using LC with UV detection. It is simple, sensitive and reproducible and the range of linearity of the response is very wide.

EXPERIMENTAL

Instrumentation

A Waters system (Millipore, Chromatography Division, Milford, MA, USA) was employed, consisting of a Model 510 high-pressure pump, a

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U6K injector and a Model 991 photodiode-array detector set at 305 nm connected to a NEC APC IV microcomputer (NEC Information Systems, Boxborough, MA, USA) using the Model 991 software.

Reagents

Zopiclone was obtained from Theraplix, Rhône Poulenc and hydroquinidine chlorhydrate from Roussel Uclaf (Paris, France). Solvents were of chromatographic grade. Dichloromethane, methanol and tetrahydrofuran (THF) were purchased from SdS (Peypin, France), and trimethylamine (TMA) (45% aqueous solution) and acetonitrile from Merck (Darmstadt, Germany). Stock standard solutions in methanol (1 mg/ml) were prepared and stored at 4°C for one month. Working standard solutions were prepared by dilution with distilled water.

Extraction procedure

Plasma (0.5–1 ml) or zopiclone standard (10–1000 ng/ml) in plasma, 250 ng of hydroquinidine as internal standard, phosphate buffer (pH 8) (1 ml) and dichloromethane (6 ml) were mixed. After centrifugation at 1000 *g* for 10 min, the organic phase was separated and evaporated to dryness under a stream of nitrogen and the residue was dissolved in 50 μl of mobile phase before being chromatographed.

Chromatography

Volumes of 20 μl were injected into the liquid chromatograph. The mobile phase consisted of acetonitrile–methanol–THF–TMA (10 mmol/l) adjusted to pH 2.5 with phosphoric acid (15:5:2:78, v/v) at a flow-rate of 1 ml/min. The chromatographic separation was performed at room temperature using a 15 cm × 4.6 mm I.D. C_{18} reversed-phase Ultrasphere ODS column, particle size 5 μm (Beckman Instruments, Altex Division, San Ramon, CA, USA).

RESULTS AND DISCUSSION

The retention times of the internal standard and the zopiclone were 3.3 and 4.6 min, respectively (Fig. 1). The calibration graph [ratio of peak height of zopiclone to that of the internal standard (y) *versus* zopiclone concentration (x)] was linear up to 1000 ng/ml ($y = 0.01x + 0.086$; $r^2 = 0.999$). This calibration range was chosen because for toxicokinetic studies we expected zopiclone concentrations exceeding the therapeutic plasma concentration of 20–80 ng/ml [10]. This range covers all clinical situations.

The limit of detection was 5 ng/ml. The recovery of extraction was 98 ± 4% (n = 11). The precision of the method was determined using quality control samples (Table I). The mean coefficients of variation of peak-height ratio to the internal standard were <7%. The accuracy, calculated by determining four concentrations of zopiclone (50, 200, 500 and 1000 ng/ml), was 1.6–7.7% (Table II).

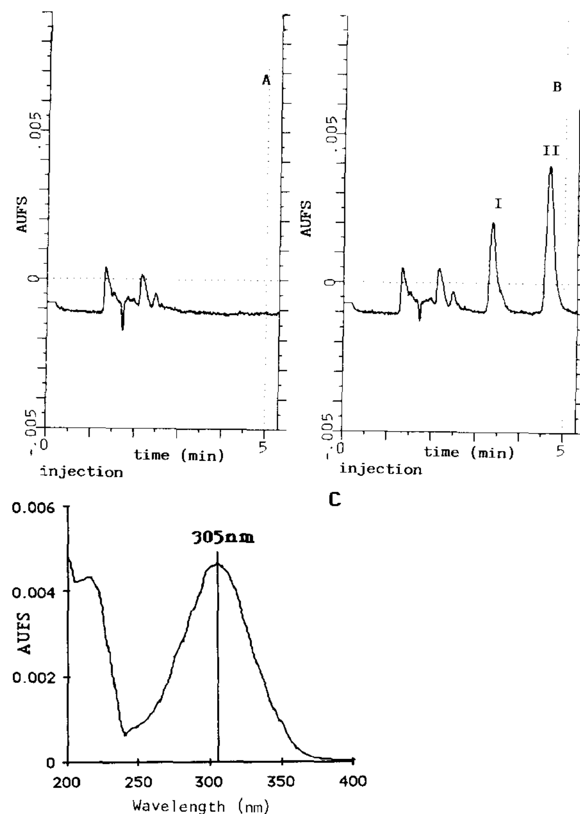

Fig. 1. (A) Chromatogram of an extract of blank plasma; (B) chromatogram of an extract of 1 ml of plasma containing 250 ng of hydroquinidine (I) and 100 ng of zopiclone (II); (C) zopiclone spectrum.

TABLE I

PRECISON OF THE METHOD

Concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	n
Within-run		
92 ± 6.1	6.6	10
519 ± 19.4	3.7	10
1010 ± 29.3	2.9	15
Between-run		
90 ± 5.8	6	6
250 ± 26	5	6

Interference studies showed that most benzodiazepines were not detectable at 305 nm; the retention times of quinidine and quinine were 2.9 and 3.1 min, respectively, and those of tricyclic antidepressants, phenothiazines and zolpidem were longer than 5 min. Zopiclone and the internal standard peak purity could be studied using the diode-array detector.

The method described was applied to study the toxicokinetics of zopiclone in a case of acute poisoning. A 25-year-old man had ingested 300 mg of the drug in an attempt at suicide. The time interval between the act and the arrival at hospital was 3.5 h and the clinical picture was sleepiness. The treatment consisted of gastric lavage and Anexate (flumazenil, Roche). The evolution was good. Volumes of 5 ml of blood were collected in heparinized Vacutainer tubes (Becton Dickinson) at time 4.5, 5.5, 6.5, 11, 16 and 23 h after the dose. The plasma was separated, frozen and stored at −20°C until assayed. The maximum

TABLE II

ACCURACY OF THE DETERMINATION OF ZOPICLONE IN PLASMA

Added (ng/ml)	Mean found (ng/ml)	Coefficient of variation (%)	n
50	51	7.7	23
200	202	5.5	21
500	504	3.4	21
1000	1000	1.6	21

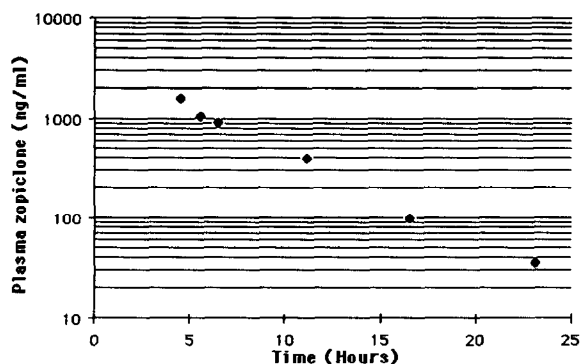

Fig. 2. Plasma concentration *versus* time profile of zopiclone after oral ingestion of 300 mg in a single dose in an attempt at suicide.

plasma concentration was 1600 ng/ml. The pattern over the period from 4.5 to 23 h after dosing was consistent with first-order elimination from a one-compartment model. The plasma elimination half-life was 3.5 h (Fig. 2). This value is in agreement with the average half-life of 5 h determined in healthy volunteers with a 7.5-mg dose [2]. Work is in progress to study the toxicokinetics in cases of acute zopiclone intoxication.

In conclusion, the proposed method is rapid, linear over a wide range, sensitive and reproducible. With regard to its analytical criteria, it is valuable for toxicokinetic and pharmacokinetic studies and for monitoring zopiclone.

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