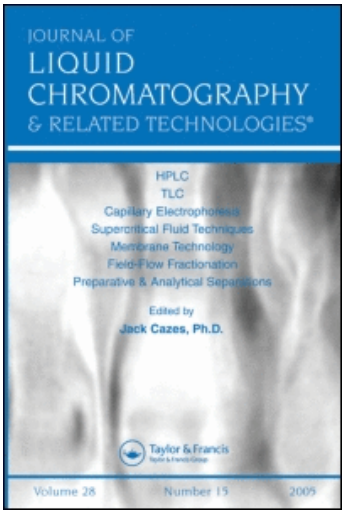

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ZOPICLONE AND ZOLPIDEM QUANTIFICATION IN HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH PHOTO-DIODE-ARRAY DETECTION

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ABSTRACT

A rapid and selective high performance liquid chromatography method for the simultaneous quantification of zopiclone and zolpidem has been developed. Signals are monitored by a photodiode-array detector with a main 305 nm wavelength and a bandwidth of 10 nm. After one step liquid phase extraction, samples of 100 µL are injected into a 5 µm ODS-2 column (30 cm x 4.6 mm I.D.). Drugs are eluted with a mobile phase containing potassium dihydrogen phosphate buffer, 0.01 M, methanol and tetrahydrofuran (30: 65: 5, V/V/V). Retention times of zopiclone and zolpidem are, respectively, 4.5 min and 5.5 min. The procedure has the necessary sensitivity and precision for pharmacokinetic studies (within day coefficients of variation < 7.1% and between-day coefficients of variation < 12.1 %). The linearity and the rapidity of this method are especially attractive for toxicological quantification in emergency toxicology.

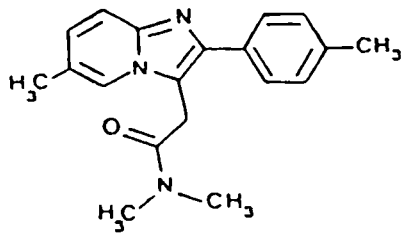

Figure 1. Structure of zolpidem.

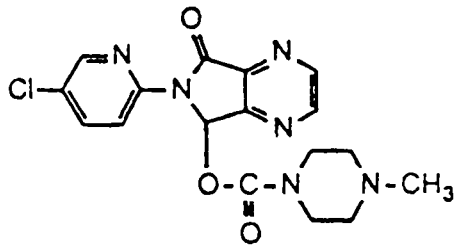

Figure 2. Structure of zopliclone.

INTRODUCTION

Zopiclone and zolpidem are two hypnotics which are widely prescribed, given their rapid onset of action and short elimination half-life. Zolpidem (Fig. 1) is an imidazopyridine agonist of the $GABA_A$ ω1 receptors,[1] whereas zopiclone (Fig. 2) is a cyclopyrolone which presents equal affinity with ω1, ω2 and ω3 receptors.[2]

As benzodiazepines, both compounds present anticonvulsant, myorelaxant, anxiolytic and sedative properties[3,4] but they may produce less dependence than benzodiazepines.[1,2] Nowadays, they are often involved in voluntary intoxications[5,6,7] and experience reveals that they could induce serious side effects.[8,9] Several analytical methods have been proposed for the determination of zopiclone or zolpidem: liquid chromatography (LC) methods using ultraviolet (UV) detection,[7,10,11] fluorimetric detection,[12-17] or diode array detection (DAD),[18] and gas chromatography (GC) methods equipped with nitrogen-phosphorus detection,[19-21] or electron capture detection for zopiclone.[22]

This paper presents a method for the rapid detection and quantification of both zopiclone and zolpidem, suitable for toxicological or pharmacokinetic studies.

MATERIALS

Apparatus

The liquid chromatographic system consisted of a constant flow-rate pump (model 110 A, Beckman Instruments, Germany), a sample injector (Rheodyne, Berkeley, CA, USA), a 5 µm particle-size C_{18} Spherisorb ODS-2 column, 30 cm x 4.6 mm I.D., (Interchrom, Montluçon, France) connected to a multiwavelength photodiode-array detector (DAD) (Waters Chromatography, Milford, USA) with a main sample wavelength of 305 nm and a bandwidth of 10 nm. Zopiclone and zolpidem spectra were previously stored in the library. The identification of each product was based on the comparison of its retention time and unknown spectrum to the different spectra stored in the library. The software calculated a fit value (degree of similarity), a degree of purity ranging from 0 to 1000, and finally identified the compound being as one of those stored in the library. The time window and the fit threshold were set at ±0.02 min and 900/1000, respectively.

Reagents

The various compounds used were kindly provided by manufacturers: zopiclone base and zolpidem hemitartrate from Rhône Poulenc Rorer (Vitry sur Seine, France). Ethanol RP Normapur, dichloromethane RP Normapur, ethyl acetate RP Normapur, orthophosphoric acid RP Normapur and tetrahydrofuran were purchased from Prolabo (Paris, France), and hexane Pestipur grade from SDS (Valdonne Peypin, France). They were of high grade of purity. Methanol, triethylamine and acetonitrile were HPLC grade, potassium dihydrogenphosphate was analytical grade (Merck, Darmstadt, Germany). Solutions at 100 mg/L of zopiclone and zolpidem were prepared monthly in acetonitrile and stored at -20 °C. Appropriate ethanolic dilutions were made each day to obtain solutions at 1 mg/L.

METHOD

Procedure

The mobile phase was a mixture of potassium dihydrogen phosphate buffer, 0.01M, methanol and tetrahydrofuran (30:65:5, V/V/V). At first, the buffer was adjusted to pH 2.6 with orthophosphoric acid, then 0.1% of triethylamine was added to obtain symmetric peaks.

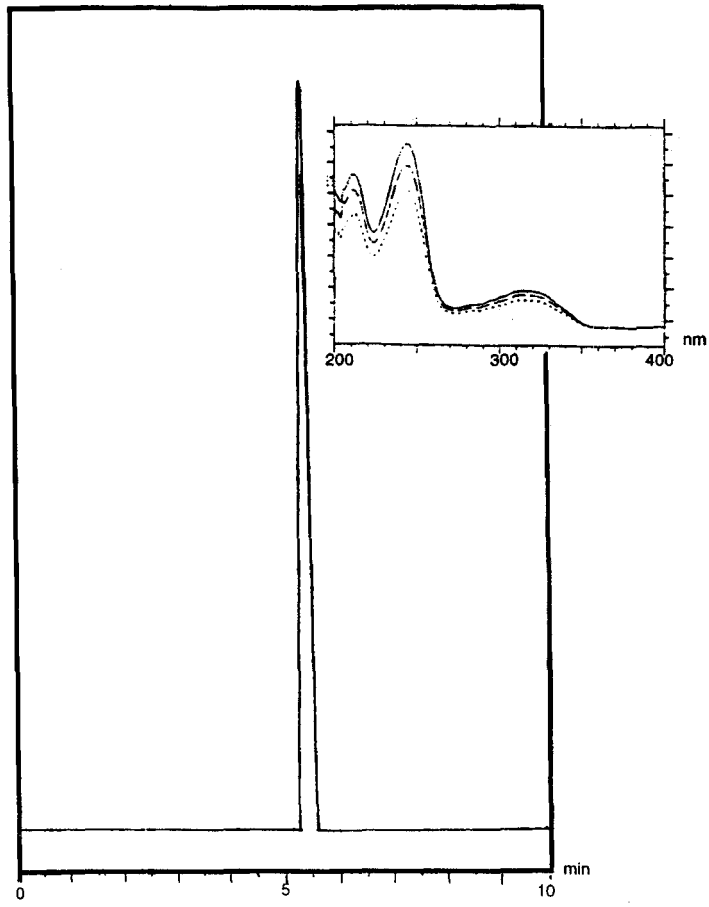

Figure 3. Chromatogram and UV spectrum of a sample containing 100 ng/mL of zolpidem.

The mobile phase was filtered through a 0.45 μm Millipore filter (Millipore, Bedford, MA, USA) and degassed in an ultrasonic cuve (Prolabo, Paris, France). The chromatographic separation was performed at ambient temperature, flow rate 0.8 mL/min.

Two mL of plasma, 2 mL of carbonate buffer, pH 9.2, and 4 mL of hexane/dichloromethane (4 : 3, V/V) were mixed on a horizontal mechanical agitator in a 15 mL centrifuge tube. After a period of centrifugation (10 min, 3000 rpm), the upper organic phase was transferred into a Reacti-Vial and evaporated to dryness under a stream of air.

Table 1

Within-Day Precision of Zopliclone and Zolpidem (n=10)

Theoretical Concentration ng/mL	Zolpidem Mean (± SD) ng/mL	Zolpidem CV (%)	Zopiclone Mean (± SD) ng/mL	Zopiclone CV (%)
50	53.77 ± 2.13	3.9	49.37 ± 3.21	6.5
100	103.39 ± 3.8	3.6	98.19 ± 3.81	3.9
200	198.99 ± 3.70	1.9	192.75 ± 13.70	7.1

Linear regression line of zolpidem: $y = 0.968x + 5.537$ ($r = 0.998$)
Linear regression line of zopliclone: $y = 0.945x + 3.526$ ($r = 0.994$)

Table 2

Between-Day Precision of Zopiclone and Zolpidem (n=10)

Theoretical Concentration ng/mL	Zolpidem Mean (± SD) ng/mL	Zolpidem CV (%)	Zopiclone Mean (± SD) ng/mL	Zopiclone CV (%)
50	47.43 ± 5.76	12.1	49.39 ± 5.47	11.0
100	99.57 ± 7.77	8.8	97.99 ± 4.71	4.8
200	195.16 ± 15.24	7.8	193.06 ± 19.03	9.8

Linear regression line of zolpidem: $y = 0.981x - 0.393$ ($r = 0.986$)
Linear regression line of zopliclone: $y = 0.932x + 1.337$ ($r = 0.994$)

The calibration was obtained by programming, daily, the diode-array detector after the injection of a sample containing 100 ng/mL of zopiclone and 100 ng/mL of zolpidem. Then, the concentration of each sample was automatically calculated by the system.

RESULTS

LC chromatograms and UV spectra obtained from the analysis of blank plasmas revealed no interference with analyses. LC chromatograms and UV spectra of a sample containing 100 ng/mL of zolpidem and a sample contai

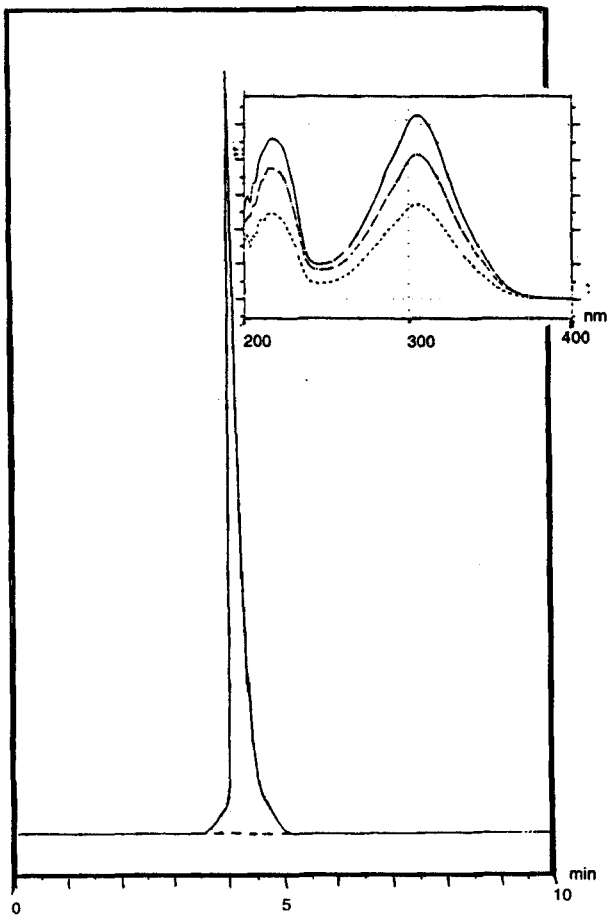

Figure 4. Chromatogram and UV spectrum of a sample containing 444 ng/mL of zopiclone.ning 444 ng/L of zopiclone are displayed in Figs. 3 and 4.

The retention times (RT) of zopiclone and zolpidem are, respectively, 4.5 min and 5.5 min. The intra-assay precision of the method was investigated by analysing 10 replicate samples of plasma containing 50, 100 and 200 ng/mL of zopiclone and zolpidem. Results are given in Table 1.

Between-day precision was calculated by the analysis of 10 replicate samples of zopiclone and zolpidem on different days. Results are given in Table 2.

Table 3

Accuracy of the Method

Theoretical Concentration ng/mL	Zolpidem Mean (± SD) ng/mL	CV (%)	Zopiclone Mean (± SD) ng/mL	CV (%)
50	54.10 ± 2.29	4.2	43.81 ± 3.53	4.2
100	103.87 ± 3.21	3.1	96.24 ± 5.58	5.8
200	204.66 ± 7.84	3.8	208.32 ± 10.1	4.8

Table 4

Overall Recovery of Zolpidem and Zopiclone Added to Blank Plasma

	Zolpidem	Zopiclone
n	10	10
Mean	88.92%	85.90%
CV	8.9%	8.9%
Sd	7.98	7.64

The accuracy was assessed by analysing five replicate samples of three concentrations of zopiclone and zolpidem on different days. Results are given in Table 3. The recovery (Table 4) was evaluated by comparing concentrations after the injection of series of samples of both compounds with, and without, extraction.

This method was linear over the ranges 20-1300 ng/mL for zolpidem and 20-1500 ng/mL for zopiclone. The limits of linearity were evaluated graphically after spiking increasing concentrations of zopiclone and zolpidem.

The instrumental limits of detection were determined by spiking extracts with decreasing concentrations of zopiclone and zolpidem until a response equivalent to three times the background was obtained. The limit of detection is 20 ng/mL for both compounds.

The examination of the blank plasma chromatogram revealed no interference with biological blood constituents. Table 5 gives retention times of psychoactive drugs which could be associated during treatment or intoxications.

Table 5

Interferences: Retention Times (min) of Several Tested Compounds

DCI	RT (min)
Acepromazine	>20
Alprazolam	>20
Amisulpiride	>20
Amitriptyline	>20
Bromazepam	5.67
Clobazam	6.33
Clotiazepam	9.31
Cyamemazine	>20
Desipramine	>20
Diazepam	10.34
Flunitrazepam	6.42
Haloperidol	>20
Imipramine	>20
Levomepromazine	>20
Lorazepam	5.73
Lormetazepam	8.41
Midazolam	10.63
Niaprazine	5.39
Nitrazepam	6.58
Nortriptyline	3.47
Prazepam	17.30

DISCUSSION

Isocratic elution was chosen for the described experiment since it provided a good separation of both compounds and required a short analysis time (RT < 6 min). We optimized the mobile phase proposed by Tracqui[18] by the addition of a small amount of triethylamine (0.1%), so we obtained shorter retention times and a symmetrical peak of zopiclone allowing accurate peak area and drug concentration calculations. As Boniface,[10] we have checked that the addition of higher percentages of triethylamine did not improve the zopiclone peak shape.

Advantages of the proposed method consisted in the simplicity of the procedure (one step liquid extraction), the rapidity of the analysis (RT <6 min), and the high specificity, which afforded a great safety for the identification of each compound: retention time and UV spectra were both taken into account for the identification.

The precision of the method was, at its best, around the median values and we observed that coefficients of variation often slightly increased for very low and high concentrations.

Moreover, the limit of detection (20 ng/mL) of both compounds was sufficient for pharmacokinetic studies, since the ranges of plasma concentrations of zopiclone and zolpidem are, respectively, 20-80 ng/mL[23] and 50-220 ng/mL[24] after orally therapeutic doses. The linearities of the method (20-1300 ng/mL for zolpidem and 20-1500 ng/mL for zopiclone) were consistent with the quantification of each compound after an acute poisoning episode.

Kennel[20] compared several organic solvents and different pH's for zopiclone extraction and obtained low and inconsistent recoveries except with chloroform at pH 6.6 (recovery: 77%). A reduction of the hexane proportion and the addition of dichloromethane at pH 9.2 were shown to be preferable in terms of recovery and reproductibility. Hexane/dichloromethane (4:3, V/V) was chosen as the extracting solvent, since it was little prone to emulsion formation and allowed good recovery (> 85%).

CONCLUSION

The method we suggest presents three major advantages: satisfactory precision, rapidity of the analysis and safety for the simultaneous identification of two common hypnotics. It is particularly suitable for toxicological emergency quantification or pharmacokinetic studies.

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