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Simultaneous determination of zolpidem and zopiclone in human plasma by gas chromatography–nitrogen–phosphorus detection

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Abstract

A simple, specific and selective method for the simultaneous determination of zolpidem and zopiclone in human plasma is described. After a liquid–liquid extraction, the extract is injected into a capillary gas chromatograph with an OV-1 fused-silica column coupled to a nitrogen–phosphorus detector. The detection limits are 1 and 2 ng/ml for zolpidem and zopiclone, respectively. The method described is reproducible and linear over a range of concentrations, rendering it suitable for use for pharmacokinetic studies or toxicological evaluations. Absolute identification of the chromatographed compounds is accomplished by gas chromatography–mass spectrometry in both electron-impact and positive-ion chemical ionisation modes.

Keywords: Zolpidem; Zopiclone

1. Introduction

Zolpidem, an imidazopyridine (Fig. 1a), and zopiclone, a cyclopyrolone (Fig. 1b), are two hypnotics which patients receive just before sleeping. They are widely prescribed on account of their rapid onset of action, short elimination half-life, and few side effects [1,2]. Therapeutic oral doses are 7.5 mg for zopiclone and 10 mg for zolpidem, and the subsequent ranges of plasma levels are 20–80 ng/ml [3,4] and 50–220 ng/ml [5], respectively. Zopiclone [6] and zolpidem [7,8] are therefore more and more often involved in voluntary intoxications. Their identification in

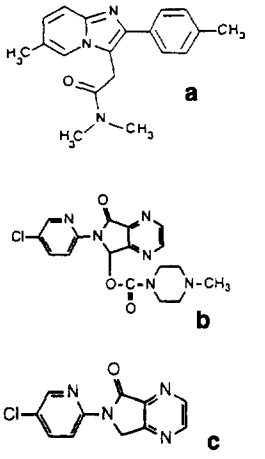

Fig. 1. Structures of (a) zolpidem, (b) zopiclone and (c) I.

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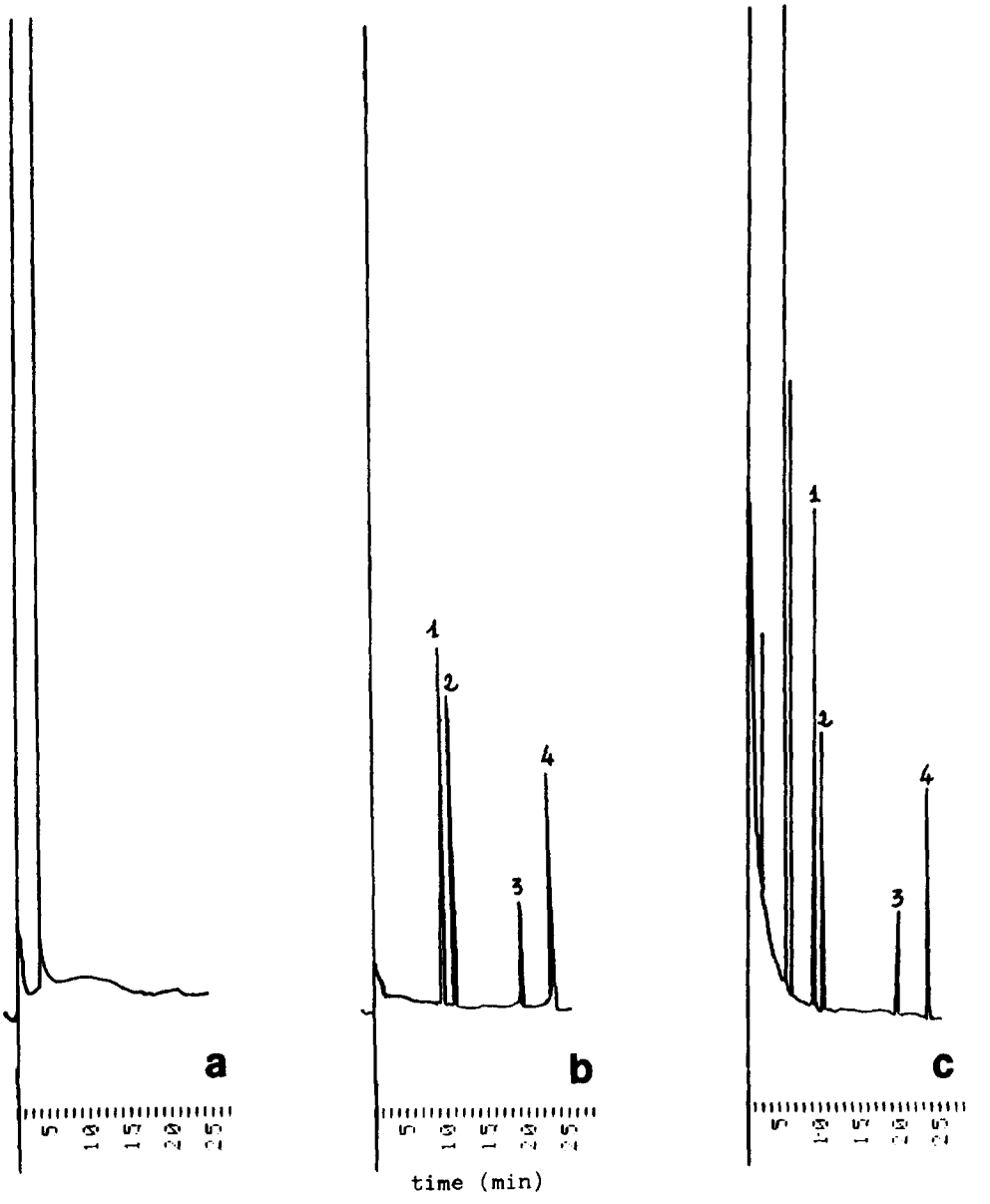

Fig. 2. Chromatograms of (a) a blank plasma, (b) an extract containing 100 ng/ml each of zolpidem and zopiclone, and (c) an extract of human plasma containing 170 ng/ml of zolpidem and 100 ng/ml of zopiclone. 1 = Zolpidem, 2 = clonazepam, 3 = zopiclone, 4 = alpidem.

routine toxicological research is not possible by immunoassays, however, and available specific assays fail commercially. Many analytical methods have been published for the determination of zopiclone based on gas chromatography (GC) [9–12] and liquid chromatography (LC) [13–18]. Other GC [9,19] and LC methods [20,21] have been described to detect zolpidem. None of the methods described, however, permits simultaneous screening of both compounds. Only Debruyne [9] reports the determination of zopiclone and zolpidem, but his method requires separative extractions and different GC conditions. To our knowledge, we describe the first method allowing

the simultaneous determination of zolpidem and zopiclone with a similar extraction procedure and common GC analysis.

Recently, a paper [11] mentioned the problem of thermal decomposition of zopiclone after GC. Therefore, we were interested to find out whether our technique detected zopiclone intact or a decomposition product (Fig. 1c, structure I). The identification of zopiclone is investigated here and a simple method of quantification is described.

2. Experimental

2.1. Standard solutions and solvents

Zopiclone and zolpidem hemitartrate were kindly supplied by Rhône-Poulenc Rorer (Vitry-sur-Seine, France). The internal standards clonazepam and alpidem were provided by Roche (Basel, Switzerland) and Synthelabo (Meudon La Forêt, France). Zopiclone and zolpidem solutions were prepared each month at a concentration of 100 mg/l in acetonitrile and were stored at −20°C; the instability of zopiclone in nucleophilic solvents has often been observed [11]. Appropriate dilutions were made daily in ethanol to obtain 1 mg/l solutions.

Ethanol, dichloromethane and ethyl acetate (RP Normapur) were purchased from Prolabo (Paris, France), hexane (99% Pestipur grade) from SDS (Valdonne-Peypin, France) and acetonitrile (gradient grade) from Merck (Darmstadt, Germany).

2.2. Instrumentation

GC was performed on a Hewlett-Packard 5890 A instrument equipped with a selective nitrogen–phosphorus detector and a split–splitless injector system. An OV-1 fused-silica capillary column, 25 m × 0.32 mm I.D., with a film thickness of 0.2 μm (Lara-Spiral, Couternon, France), was employed. The chromatograph was coupled to a D 2500 Merck data collector. The GC conditions were as follows: injector and detector temperature, 270°C; oven temperature, 235°C; injection mode, split with a splitting ratio of 15. The carrier gas was helium with a column linear velocity of 1.3 ml/min. Other flow-rates were: make-up gas, 29.7 ml/min; hydrogen, 3.4 ml/min; and air, 81.7 ml/min. The reporting integrator used peak-height ratios to calculate drug concentrations.

The confirmation of zopiclone identity was performed by a Fisons MD 800 mass selective detector interfaced with a Fisons GC 8000 gas chromatograph. The injector and interface temperature was 270°C. The gas chromatograph operated isothermally at 200°C for 5 min followed by 10°C/min programming to 280°C, and was kept at this temperature for 5 min for every analysis. A fused-silica capillary column, JW DB-5 MS 5% phenyl silicone, 15 m × 0.25 mm I.D., with 0.25 μm film thickness, was employed. Methane was used for chemical ionisation (CI) analysis. These conditions were also tested on the Hewlett-Packard 5890 A gas chromatograph and provided similar chromatograms.

2.3. Extraction procedure

Internal standard solutions (alpidem and clonazepam) were prepared at concentrations of 1 mg/l by dilution with ethanol and put into 20-ml pyrex tubes. Human plasma (1 ml), 300 μl of 1 mg/l alpidem solution, 400 μl of 1 mg/l clonazepam solution, 2 ml of carbonate buffer (pH 9.2), and 4 ml of an organic mixing solution containing hexane–dichloromethane (4:3, v/v) were added. The mixture was shaken mechanically for 10 min and centrifuged for 10 min at 2200 *g*. The upper organic phase was transferred to a Reacti-Vial and evaporated to dryness at ambient temperature. The residue was dissolved in 50 μl of ethyl acetate and 2 μl of this solution were injected. Drug-free plasma was analysed in the same way as a control.

2.4. Calibration

Concentrations of zolpidem and zopiclone were determined from calibration graphs. Graphs of the peak heights of zolpidem and zopiclone relative to those of their internal

Table 1
Intra-assay precision ($n = 10$)

Theoretical concentration (ng/ml)	Zolpidem[a]		Zopiclone[b]	
	Ratio[c] (mean ± S.D.)	C.V. (%)	Ratio[c] (mean ± S.D.)	C.V. (%)
50	0.319 ± 0.024	7.5	0.251 ± 0.020	7.9
100	0.703 ± 0.030	4.2	0.363 ± 0.029	7.9
200	1.758 ± 0.042	2.4	0.769 ± 0.036	4.6

[a] Linear regression line of zolpidem: $y = 0.0096x - 0.201$ ($r = 0.995$).
[b] Linear regression line of zopiclone: $y = 0.0035x + 0.048$ ($r = 0.992$).
[c] Ratio of peak-height response of the analyte to peak-height response of the internal standard.

standards against drug concentration were displayed over the ranges 1–400 ng/ml for zolpidem, and 2–400 ng/ml for zopiclone.

3. Results

Typical chromatograms obtained from the analysis of a blank plasma, of samples each containing 100 ng/ml of both zolpidem and zopiclone, and of the plasma of a patient for toxicological research, are displayed in Fig. 2a, b and c, respectively.

The retention times (tR) were 10.02 min for zolpidem, 10.72 min for clonazepam, 19.59 min for zopiclone and 23.02 min for alpidem.

3.1. Method validation parameters

Intra-assay and inter-assay precision

Within-run precision was calculated from repeated analysis ($n = 10$) during one working day. Intra-assay precision was calculated for three concentrations of zolpidem and zopiclone. Results are given in Table 1.

Day-to-day precision was calculated from repeated analysis of quality control samples on ten successive working days. Table 2 shows day-to-day precision for the same concentrations of zolpidem and zopiclone.

Recovery

Recovery was calculated by comparing drug/internal standard peak-height ratios with and

Table 2
Day-to-day precision ($n = 10$)

Theoretical concentration (ng/ml)	Zolpidem[a]		Zopiclone[b]	
	Ratio[c] (mean ± S.D.)	C.V. (%)	Ratio[c] (mean ± S.D.)	C.V. (%)
50	0.477 ± 0.054	11.3	0.240 ± 0.031	12.9
100	0.911 ± 0.067	7.3	0.403 ± 0.042	10.4
200	1.817 ± 0.088	4.8	0.833 ± 0.069	8.2

[a] Linear regression line of zolpidem: $y = 0.0089x + 0.0237$ ($r = 0.992$).
[b] Linear regression line of zopiclone: $y = 0.0039x + 0.036$ ($r = 0.975$).
[c] Ratio of peak-height response of the analyte to peak-height response of the internal standard.

Table 3
Mean overall recoveries of zolpidem and zopiclone ($n = 10$)

Compound	Recovery (mean ± S.D.)	C.V. (%)
Zolpidem	89.82 ± 7.97	8.9
Zopiclone	82.59 ± 7.64	9.3

without extraction. The mean overall recoveries are given in Table 3.

Accuracy

The accuracy of the method was assessed by carrying out five replicate analyses of three concentrations of zolpidem and zopiclone on different days. Results are given in Table 4.

Limits of detection and linearity

The limits of detection were determined by spiking plasma with decreasing concentrations of zopiclone and zolpidem until a response equivalent to three times the background noise was obtained. Under the conditions adopted, the limits of detection based on a 1-ml plasma sample were 1 ng/ml for zolpidem and 2 ng/ml for zopiclone.

Typical plots of the drug/internal standard peak-height ratio versus plasma standard concentrations were linear over the range 0.001–2.0 μg/ml for zolpidem and 0.002–1.3 μg/ml for zopiclone.

Table 5
Interferences: rINN and retention times of the compounds tested

rINN[a]	Retention time (min)
Amitriptyline	3.10
Nortriptyline	3.20
Imipramine	3.27
Desipramine	3.39
Prometazine	3.62
Oxazepam	3.92
Lorazepam	4.62
Diazepam	5.10
Clotiazepam	5.67
Levomepromazine	5.89
Clobazam	5.99
Bromazepam	6.61
Midazolam	6.68
Flunitrazepam	6.72
Cyamemazine	6.78
Prazepam	7.54
Lormetazepam	7.97
Acepromazine	8.66
Nitrazepam	9.04
Haloperidol	12.19
Alprazolam	14.25
Tianeptine	S
Fluvoxamine	S
Amisulpiride	S

S = Retention time >24 min.
[a] rINN = Recommended international nonproprietary name modified.

Plasma interference and selectivity

The blank plasma after extraction consistently contains no interfering peak (Fig. 2a).

Table 5 shows retention times of 24 psychoac-

Table 4
Accuracy ($n = 5$)

Concentration added (ng/ml)	Zolpidem		Zopiclone	
	Concentration found (mean ± S.D.) (ng/ml)	C.V. (%)	Concentration found (mean ± S.D.) (ng/ml)	C.V. (%)
50	53.00 ± 1.22	2.3	48.60 ± 4.39	9.0
100	99.00 ± 1.00	1.0	98.40 ± 4.93	5.0
200	200.20 ± 0.44	0.2	202.80 ± 4.43	2.2

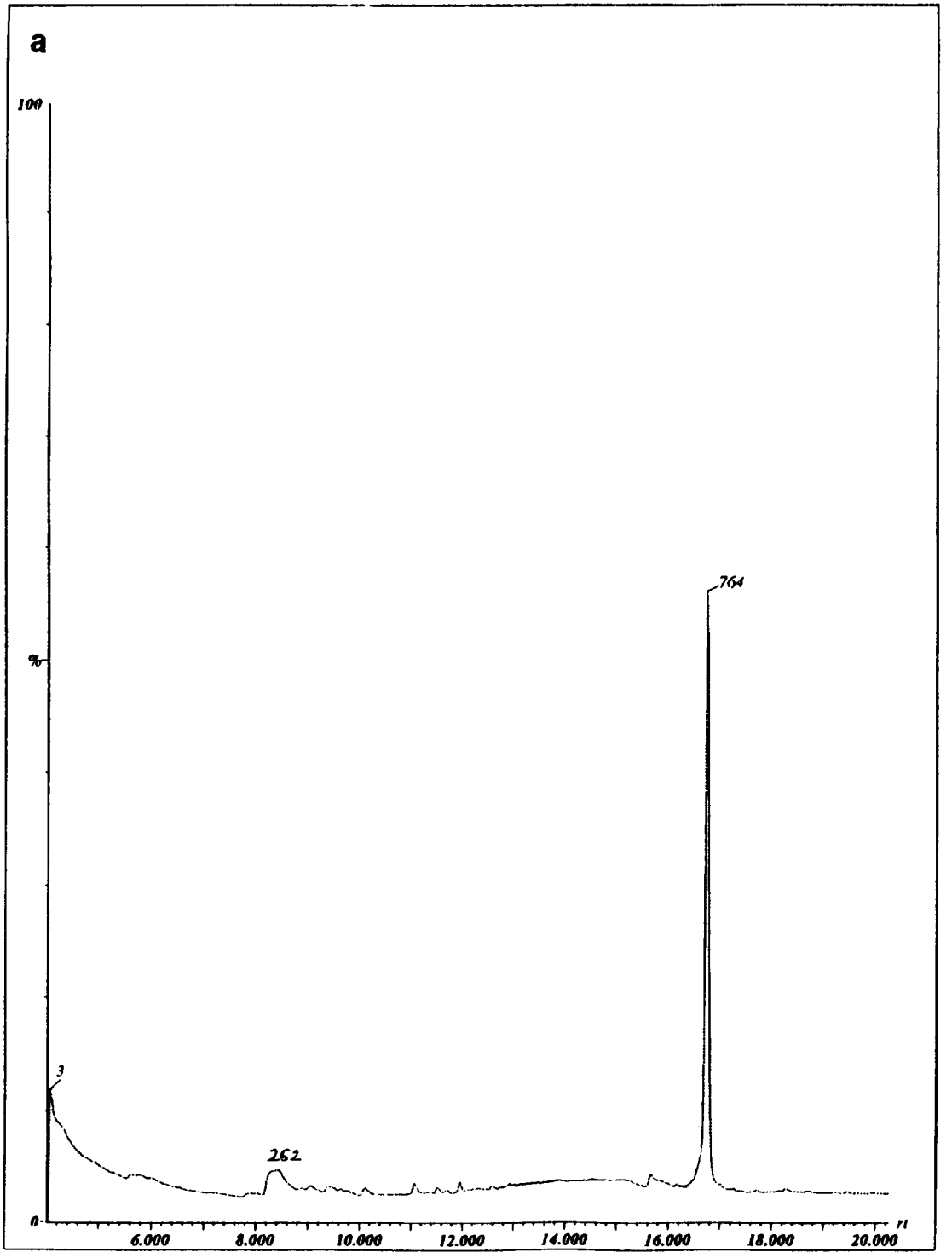

Fig. 3. (a) Total ion current (TIC) chromatogram and (b) mass spectra of zopiclone recorded in electron-impact (EI) mode. GC–MS conditions: temperature, 200 to 280°C; rate, 10°C/min; initial time, 5 min; final time, 5 min; scanning range, $m/z = 40$–450. (a) Retention time in min.

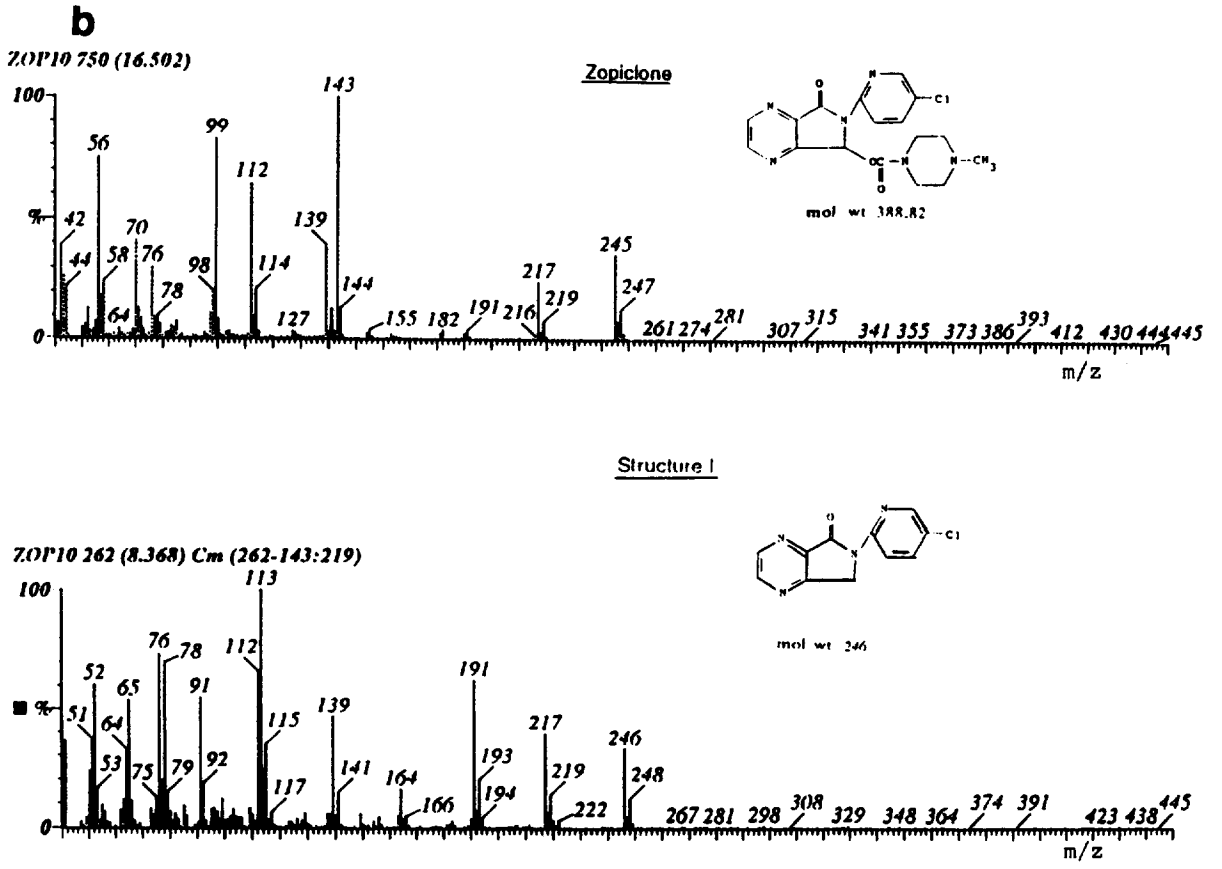

Fig. 3. (*Continued*)

tive drugs which could be present during zolpidem or zopiclone intoxication or its treatment. None of these drugs interferes with zolpidem, zopiclone or the two internal standards.

3.2. Analysis

The main data obtained from the total ion current (TIC) chromatogram and the mass spectra in GC–MS analysis are represented in Figs. 3 and 4.

Fig. 3a and b gives the TIC and mass spectra of zopiclone and structure I in the electron-impact (EI) mode. Equivalent mass spectra recorded in the positive-ion chemical ionisation (PCI) mode are displayed in Fig. 4.

4. Discussion

Our method allows the simultaneous screening and quantification of zolpidem and zopiclone over a therapeutic and toxicological range of concentrations. However, previous investigations [11] have demonstrated that injection-port temperature, liner conditions and different injection modes could interfere with zopiclone quantification. Our purpose was to determine the identity of each chromatographic peak in a GC–MS analysis on a Fisons MD 800 gas chromatograph.

The main data obtained from the total ion current (TIC) chromatogram and mass spectra of plasma samples spiked with zopiclone are presented in Figs. 3 and 4. In the EI mode, the TIC displays a main peak at t_R 16.50 min and a peak

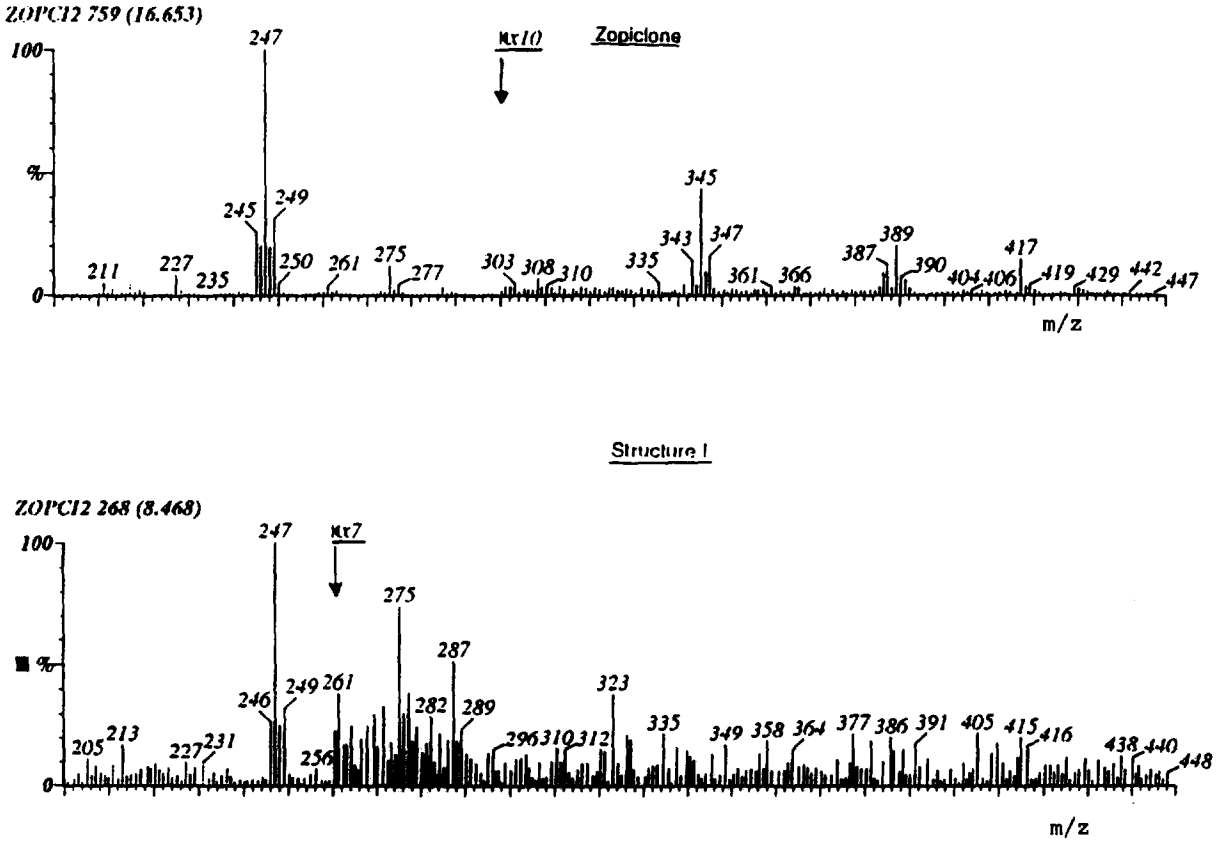

Fig. 4. Mass spectra of zopiclone and structure I recorded in positive-ion chemical ionisation (PCI) mode (same GC–MS conditions as in EI mode). Methane used as reagent gas.

trace at t_R 8.36 min. Study of the peak trace occurring at t_R 8.36 min confirms the identity of a compound with the I structure ($M_r = 246$) described by Boniface [11]. In the EI mode, the mass spectrum exhibits a base peak at 113 and a molecular ion at 246. In the PCI mode, quasimolecular ions $(M + 1)^+$ at $m/z = 247$ and $(M + C_2H_5)^+$ at $m/z = 275$ are observed. The presence of ions at 247 and 249 at a ratio of 3:1 is a typical pattern for a compound with a chlorine substituent.

According to EI and PCI mass spectra of the major peak at t_R 16.50 min, we confirm the identity of intact zopiclone ($M_r = 388$). In the EI mode, the mass spectrum does not exhibit a molecular ion at $m/z = 388$; only fragment ions at 245 and 247 are observed. But in the PCI mode, quasimolecular ions $(M + 1)^+ = 389$ and $(M + C_2H_5)^+ = 417$ are observed effectively.

No further compounds of thermal rearrangement of zopiclone were detected in either our GC–MS or our GC–NPD detection run.

Finally, our GC–MS analysis proves that, under the GC conditions adopted, zopiclone undergoes only very slight degradation without quantitative incidence.

The rather different retention times of zopiclone and zolpidem strongly suggest that, when the two products are to be quantified simultaneously, two internal standards should be included. In this case, if zolpidem alone were being studied, the analysis run time could be reduced. Clonazepam and alpidem were chosen as internal standards for zopiclone and zolpidem, respective-

ly: clonazepam is a benzodiazepine, used only as an adjuvant in epilepsy treatment. It is rarely involved in voluntary intoxications and its t_R is very close to that of zolpidem. Alpidem was withdrawn from the market in 1993 because of its amnesic effects.

Kennel et al. [10] compared several organic solvents and different pH values for zopiclone extraction, and obtained only low and inconsistent recovery except with chloroform at pH 6.6 (recovery: 77%). A decrease in the proportion of hexane and the addition of dichloromethane at pH 9.2 were shown to be preferable in terms of recovery and reproducibility. Hexane–dichloromethane (4:3, v/v) was chosen as the extracting solvent, since it was hardly prone at all to emulsification and allowed good recovery (zopiclone, 82.59%; zolpidem, 89.82%).

5. Conclusions

Advantages of the proposed method include rapid determination of the two drugs and a common extraction procedure and GC conditions, which allow the simultaneous determination of zolpidem and zopiclone in human plasma. The sensitivity and linearity of the method render it suitable for rapid toxicological screening as well as for precise quantification in pharmacokinetic studies.

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