CASE REPORT

Robert C. Meatherall,[1] *Ph.D.*

Zopiclone Fatality in a Hospitalized Patient

REFERENCE: Meatherall RC. Zopiclone fatality in a hospitalized patient. J Forensic Sci 1997;42(2):340–343.

ABSTRACT: The death of a 72-year-old man is described who overdosed himself while in hospital. The man was being treated for lung cancer and ingested 90 mg of zopiclone in a suicide attempt. He died between 4 and 10 h after the ingestion. Zopiclone, quantitated by GC-MS in the femoral blood, cardiac blood, vitreous humor, urine and bile was found to be 254, 408, 94, 7,330, and 114,700 ng/mL, respectively. Considering the man's weakened physical condition, 90 mg could represent a minimum lethal zopiclone dose.

KEYWORDS: forensic science, forensic toxicology, zopiclone, gas chromatography-mass spectrometry, overdose, fatality

Zopiclone is a short acting sedative-hypnotic agent used to treat insomnia (1,2). Although it has hypnotic, anxiolytic, and anticonvulsant properties similar to the benzodiazepines, its chemical structure is unrelated. The mechanism of action is nevertheless through the benzodiazepine receptor.

Case History

The 72-year-old man was admitted to hospital, 3 weeks prior to his death for treatment of lung cancer and peptic ulcer. As an outpatient, he had previously received radiation therapy and chemotherapy. Now the cancer was thought to have metastasized.

Progressive generalized weakness was apparent. He was prescribed five oral medications: 20 mg furosemide, daily, 5 mg morphine elixir, four times daily, 4 mg dexamethasone, three times daily, 10 mg metoclopramide, four times daily, and 15 mg zopiclone at bedtime. At 6:00 a.m. on the day he died, nursing staff found him sedated with a variety of his pills scattered on the bed. Apparently he had been hoarding some of the pills during his hospital stay. He was somewhat arousable and when questioned, said he took 12 zopiclone pills. He died 4 h later. No treatment was administered.

At autopsy the man was found to have small cell carcinoma of the right lung with metastases to mediastinal lymph node and left adrenal. There was pulmonary fibrosis with occlusive emboli. He had advanced calcific systemic arteriosclerosis. Blood, urine, bile, and vitreous fluid were collected for drug analysis.

[1]Toxicologist, Department of Biochemistry, St. Boniface General Hospital, Winnipeg, Manitoba, Canada R2H 2A6.

Received 13 May 1996; accepted 12 June 1996.

Methods

A general drug screen was performed on the urine specimen by gas chromatography-mass spectrometry. Basic and acid-neutral extracts were obtained using methods based on those described by Foerster et al. (3,4). Zopiclone and metoclopramide were identified. The urine was further screened for amphetamines, barbiturates, benzodiazepines, benzoylecgonine, cannabinoids, opiates and phencyclidine with EMIT II (Syva, San Jose, CA) enzyme immunoassays. Only the opiates screen was positive. Alcohols were screened by gas chromatography. None were detected in blood, urine, and vitreous humor. Zopiclone, N-desmethylzopiclone, and zopiclone-N-oxide were kindly supplied by Rhone-Poulenc Rorer (Montreal, Canada). Metoclopramide HCl was obtained from Sigma Chemical Co. (St. Louis, MO) and the internal standard p-Cl-disopyramide was obtained from Hoechst-Roussel (Montreal, Canada).

Stock solutions of zopiclone and metabolites were prepared at 1 mg/mL in acetonitrile. It was previously demonstrated (5) that zopiclone decomposes when dissolved in methanol or ethanol but is stable in acetonitrile for at least 2 months. Metoclopramide, 1 mg/mL stock solution, was prepared in methanol.

Combined zopiclone and metoclopramide were prepared in blood and urine at 2,000 ng/mL. They were further diluted to 1500, 1000, 500, 200, 100, and 0 ng/mL. Blood and bile unknowns were compared to the standard curve prepared in blood whereas urine and vitreous unknowns were compared to the standard curve prepared in urine.

The following were combined in a 150 by 15 mm extraction tube: 1 mL sample, 10 μL of internal standard (p-Cl-disopyramide 100 μg/mL in methanol), 800 μL of 1 M $NaHCO_3$ pH 9, 4 mL of methyl-tert-butylether and 2 mL methylene chloride. The tubes were closed with teflon lined screw caps, mixed on a rotating wheel for 15 min, then centrifuged for 5 min at 1000 Xg. The organic layer was transferred to 5 mL conical tubes and evaporated to residue in a 40°C sand bath under a nitrogen stream. The residue was dissolved in 100 μL of 0.1 N HCl and washed five times with 250 μL portions of methyl-tert-butyl-ether. Each time the ether was vortex mixed with the aqueous layer, the tube briefly centrifuged and the ether aspirated to waste. The ether washes are required to remove zopiclone interference from cholesterol. The acidic aqueous layer was then made basic with the addition of 100 μL of 1 M $NaHCO_3$ pH 9 and extracted with 2 mL of methylene chloride by vortexing. The tube was centrifuged and the top aqueous portion aspirated to waste. The methylene chloride was dried with anhydrous sodium sulfate, and transferred to a clean 5 mL

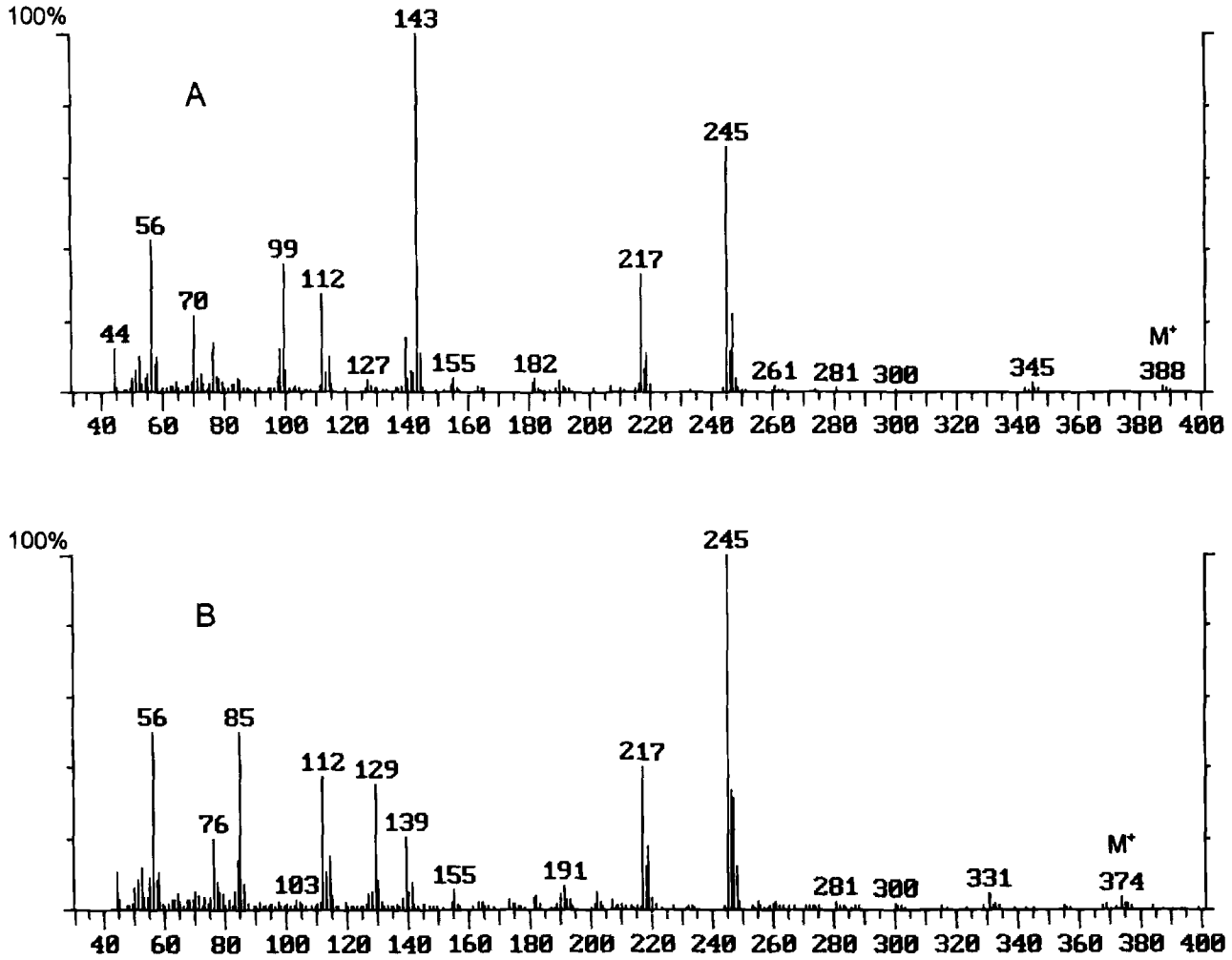

FIG. 1—*Mass spectrum of zopiclone, scan 1177* (top); *and mass spectrum of N-desmethylzopiclone, scan 1197* (bottom).

conical tube. After the addition of 100 μL of ethyl acetate, the methylene chloride was reduced to 25 μL under a stream of nitrogen.

Analysis of the extracts was done on an ITS-40 ion trap mass spectrometer controlled by Magnum software (Finnigan MAT, San Jose, CA). The capillary column was a 15 m by 0.25 mm DB-1 coated with 0.25 μm methyl silicone film (J & W Scientific, Folsom, CA). A 1 m by 0.52 mm retention gap coated with 5% phenyl 95% methyl silicone (Restek, Bellefonte, PA) was used to connect the column via a Vu-Union® (Restek) to the temperature programmable injector. Extracts were injected directly onto the retention gap. The helium carrier gas was set to flow at 45 cm/s. The oven was initially held at 85°C for 1 min, then programmed at 10°C/min to 290°C where it was held for 5 min. The injector program was the same as the oven except the initial temperature was held for 0.5 min and the program rate was 13°C/min. Electron impact spectra were collected over the 44 to 650 mass range at a 1 scan/sec rate. Peak areas of zopiclone, metoclopramide, and p-Cl-disopyramide were integrated using ions with m/z = 245, 86, and 229 respectively. The Magnum software was used to calculate peak area ratios and performed interpolations based on linear regression.

Morphine in urine was quantitated with a modification of the method described by Paul et al. (6). Morphine in blood was quantitated the same way after a protein precipitation step. After adding internal standard to 1 mL of blood in a test tube, two 1 mL portions of acetonitrile were added with vortexing to form a protein precipitate. The supernatant was decanted into a clean test tube, evaporated to about 200 μL, then brought to 1 mL with water. Two blood samples were analyzed. One without hydrolysis to give the free morphine concentration and one with hydrolysis to give the total morphine concentration.

Results

During preliminary method development, dilutions of the stock standards of zopiclone and its two metabolites were injected separately on the GC-MS. Zopiclone elutes at 1177 sec. N-desmethylzopiclone elutes 20 sec after zopiclone. Their mass spectra are shown in Fig. 1. Zopiclone-N-oxide had the same retention time and mass spectrum as zopiclone. Possibly the N-oxide metabolite was converted to zopiclone in the gas chromatograph. When urine spikes containing N-desmethylzopiclone and zopiclone-N-oxide were taken through the analysis, neither one appeared in the chromatograms. The reason for their loss was not investigated. From pharmacokinetic studies based on HPLC analysis, it is known that both metabolites are usually below detectable concentrations in plasma and blood samples but normally exceed the parent zopiclone concentration in urine following ingestion of therapeutic doses (2,7,8). In overdoses, most of the urine drug excretion is

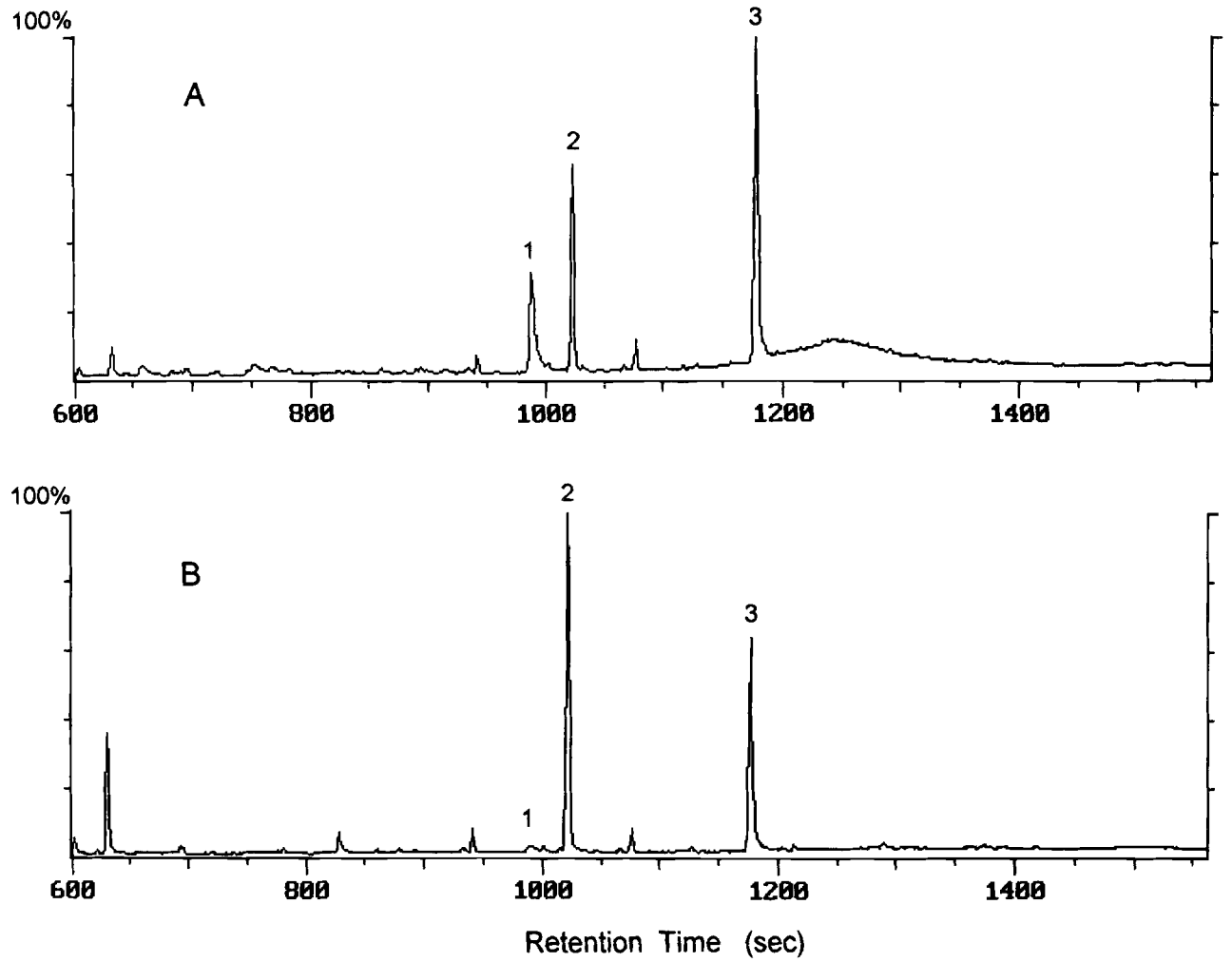

FIG. 2—*Chromatogram of a 10-fold diluted urine case specimen. Multiplied drug concentrations are zopiclone = 7,330 ng/mL, metoclopramide = 10,800 ng/mL* (top); *and chromatogram of femoral blood case specimen. Zopiclone = 254 ng/mL, metoclopramide = 72 ng/mL. Peak 1 = metoclopramide, peak 2 = internal standard, peak 3 = zopiclone.*

TABLE 1—*Zopiclone case summary.*

ng/mL	Blood		Vitreous	Urine	Bile
	Femoral	Cardiac			
zopiclone	254	408	94	7,330	114,700
metoclopramide	72	117	231	10,800	20,200
morphine					
total	51	nd	nd	6,313	nd
free	<10	nd	nd	nd	nd

nd = not done.

presumed to be in the form of the parent drug. Neither metabolite was detected in blood and liver samples from two reported fatalities using an HPLC method capable of detecting them (13).

Figure 2 shows the chromatogram of the case urine specimen. It was diluted 10 fold with water to bring the drug concentrations within range of the calibration curve. The temperature programmable injector allowed reliable quantitation of intact zopiclone. When a heated injector is used, significant thermal decomposition of zopiclone results in loss of drug and the appearance of a breakdown product called compound V which elutes at a much earlier retention time (5). The mass spectrum of zopiclone shown in Fig. 1 is identical to the late eluting peak described by Boniface et al. (5). The mass spectrum of compound V has significant ions with m/z = 246, 217, 191, 139, and 113. No early eluting peaks were found with a mass spectrum resembling compound V. The chromatogram of the femoral blood sample is also shown in Fig. 2. Zopiclone and cholesterol co-elute on a nonpolar methyl silicone DB-1 capillary column. To remove cholesterol interference, the initial extract was dried to residue, dissolved in dilute hydrochloric acid and washed 5 times with methyl-tert-butyl-ether. The acid layer was then alkalinized and re-extracted. Back-extraction of the initial organic extract into 2 mL of 0.1 N HCl gave recoveries in the order of 40% of that obtained by this method. In separate experiments, it was shown that zopiclone and metoclopramide are not lost to the methyl-tert-butyl ether during the washing procedure.

Discussion

The toxicological findings for this case are summarized in Table 1. As would be expected, the concentrations of zopiclone and metoclopromide were higher in the urine and bile than in the blood. Pounder and Davies (9) reported a zopiclone fatality in which the femoral blood, urine, and bile drug concentrations were 1,200, 10,500, and 14,100 ng/mL respectively. They also reported little

TABLE 2—*Summary zopiclone overdoses.*

Reference	Blood Zopiclone ng/mL		Other Significant Findings
Fatalities			
*	femoral	254	metoclopramide (S), morphine (S)
	cardiac	408	
12	unspecified	1,180	pentazocine = 41 μg/mL (F)
9	femoral	1,200	ethanol = 153 mg/dL
	cardiac (r)	900	
	cardiac (l)	1,200	
5	unspecified	620	trichloroethanol (F)
5	unspecified	1,160	dothiepin (F) procyclidine (F)
5	unspecified	400	temazepam (T)
5	unspecified	1,700	amylobarbitone (F)
13	femoral	3,500	quinine (T)
	carotid	3,900	
13	subclavian	1,400	ethanol = 185 mg/dL, fenfluramine (T)
Survivor			
11		1,600	blood taken 4.5 h after ingestion of 300 mg.

S = subtherapeutic.
T = therapeutic.
F = fatal.
* = this work.

postmortem redistribution between peripheral and cardiac blood. In Table 1, there are substantial differences between the femoral blood and cardiac blood concentrations of both zopiclone and metoclopramide.

In six different pharmacokinetic studies, each involving eight or more healthy subjects, the average peak plasma zopiclone concentration was between 63.5 and 69.5 ng/mL following a 7.5 mg oral dose. From the same studies, the elimination half-life ranged from 3.5 to 6.5 h (2). The deceased was thought to have ingested twelve 7.5 mg pills totaling 90 mg within 4 to 10 h prior to his death. The single dose pharmacokinetics supports this concept.

The metoclopramide did not contribute to the man's death. Repeated therapeutic administration of 20 mg oral doses of metoclopramide every 6 h resulted in typical plasma concentrations in the 200–400 ng/mL range (10). Metoclopramide in the femoral and cardiac blood were subtherapeutic, 72 and 117 ng/mL respectively. Similarly, the free and total morphine concentrations are interpretable as being subtherapeutic.

A summary of blood concentrations in reported zopiclone overdoses is shown in Table 2. There are nine fatalities and one survivor. The highest blood concentration of the survivor is higher than all but two of the fatal blood concentrations. The survivor was actively treated with gastric lavage and support (11). Presumably several hours are required after drug ingestion before death occurs. By this time, tissue distribution has occurred and the blood concentration has diminished from its peak value. Four of the nine zopiclone fatalities also had lethal blood concentrations of other drugs. An additional two fatalities had toxic blood ethanol concentrations. Two others had therapeutic concentrations of another drug; temazepam in one case, quinine in the second. In the case presented here, subtherapeutic blood concentrations of metoclopramide and morphine were found. Only 90 mg of zopiclone was ingested which probably represents a minimum lethal dose considering the man's weakened condition and his arteriosclerosis.

References

1. Imovane® product monograph. Compendium of Pharmaceutical Specialties, 1996. Pharmaceutical Association of Canada, Ottawa, Canada.
2. Goa KL, Heel RC. Zopiclone: A review of pharmacodynamic and pharmacokinetic properties and therapeutic efficacy as a hypnotic. Drugs 1986;32:48–65.
3. Foerster EH, Hatchett D, Garriott JC. A rapid, comprehensive screening procedure for basic drugs in blood or tissues by gas chromatography. J Anal Toxicol 1978;2:50–55.
4. Foerster EH, Dempsey J, Garriott JC. A gas chromatographic screening procedure for acid and neutral drugs in blood. J Anal Toxicol 1979;3:87–91.
5. Boniface PJ, Martin IC, Nolan SL, Tan ST. Development of a method for the determination of zopiclone in whole blood. J Chromatogr 1992;584:199–206.
6. Paul BD, Mell LD, Mitchell JM, Irving J, Novak A. Simultaneous identification and quantitation of codeine and morphine in urine by capillary gas chromatography and mass spectrometry. J Anal Toxicol 1985;9:222–6.
7. Liboux AL, Frydman A, Gaillot J. Simultaneous determination of zopiclone and its two major metabolites (N-oxide and N-desmethyl) in human biological fluids by reverse-phase high-performance liquid chromatography. J Chromatogr 1987;417:151–8.
8. Fernandez C, Maradeix V, Gimenez F, Thuillier A, Farinotti R. Pharmacokinetics of zopiclone and its enantiomers in Caucasian young healthy volunteers. Drug Metabolism and Disposition 1993;21:1125–8.
9. Pounder DJ, Davies JL. Zopiclone poisoning: Tissue distribution and potential for postmortem diffusion. For Sci Intl 1994;65: 177–83.
10. Bruera ED, MacEachern TJ, Spachynski KA, LeGatt DF, MacDonald RN et al. Comparison of the efficacy, safety and pharmacokinetics of controlled release and immediate release metoclopramide for the management of chronic nausea in patients with advanced cancer. Cancer 1994;74:3204–11.
11. Royer-Morrot MJ, Rambourg M, Jacob I, Bauer P, Royer RJ. Determination of zopiclone in plasma using column liquid chromatography with ultraviolet detection. J Chromatogr 1992;581:297–9.
12. VanBocxlaer J, Meyer E, Clauwaert K, Lambert W, Piette M, DeLeenheer A. Analysis of zopiclone (Imovane) in postmortem specimens by GC-MS and HPLC with diode-array detection. J Anal Toxicol 1996;20:52–4.
13. Boniface PJ, Russell SG. Two cases of fatal zopiclone overdoses. J Anal Toxicol 1996;20:131–3.

Additional information and reprint requests:
Robert C. Meatherall, Ph.D.
Department of Biochemistry
St. Boniface General Hospital
409 Tache Avenue
Winnipeg, Manitoba
Canada R2H 2A6