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Zolpidem Distribution in Postmortem Cases

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ABSTRACT: Zolpidem is the prototype of a class of sedative hypnotic drugs that are derivatives of imidazopyridine and is sold in the United States under the trade name Ambien®. Over a four-year period, zolpidem was identified in eight cases investigated by the Office of the Chief Medical Examiner, State of Maryland. Zolpidem was identified by gas chromatography-nitrogen-phosphorus detection (GC-NPD) following an alkaline extraction and was confirmed by full-scan electron impact gas chromatography/mass spectrometry. Zolpidem was quantitated by GC-NPD in all specimens received. Five of the cases presented were deaths due to drug intoxication. In three of these cases, zolpidem was an incidental finding because the drug fatalities resulted from other drugs. In the other two cases of drug intoxication, zolpidem was present in elevated concentrations and was a contributing, but not exclusive cause of the drug intoxication. The remaining three cases were deaths that were not caused by drugs. The blood zolpidem concentrations in these cases were therapeutic (0.28, 0.12 and 0.19 mg/L, respectively). In six of the eight cases where both blood and urine were analyzed, the blood concentration was higher than the urine concentration. The distribution of zolpidem into the liver and kidney failed to identify any sequestration of the drug into either specimen.

KEYWORDS: forensic science, forensic toxicology, zolpidem, Ambien, tissue distribution

Zolpidem is a non-benzodiazepine sedative-hypnotic agent which nonetheless possesses some structural similarities to this class of drugs. The structure is shown in Fig. 1. Whereas benzodiazepines bind nonspecifically to the three known benzodiazepine receptor subtypes (BZ_1, BZ_2, BZ_3), zolpidem binds specifically to the BZ_1 receptor which is responsible for sedative activity. This receptor appears in the cerebellum and in the cerebral cortex. It is sold in the United States under the trade name Ambien®. It is used as a sedative prior to surgery and as a treatment for insomnia. Minimal impairment of psychomotor functions is noted in both healthy and sleep-deprived individuals when given in doses ranging from 5 to 20 mg. Zolpidem has also compared favorably to many benzodiazepines in reduced memory loss (1,2).

Zolpidem is rapidly absorbed following oral administration, but undergoes a significant first-pass effect: the bioavailability is approximately 70% after 5 to 20 mg doses. A steady-state maximum plasma concentration of approximately 0.2 mg/L is usually seen and corresponds to the peak plasma concentration after a single dose. The elimination half-life of the drug is approximately 2 h. Like benzodiazepines, zolpidem is highly protein bound, with a free fraction less than 0.15. It is also extensively metabolized, with less than 1% of a dose appearing in the urine as unchanged drug. Metabolic routes include oxidation of the methyl group on the phenyl ring or the imidazopyridine group to produce carboxylic acids and hydroxylation of the imidazopyridine group (1,2).

The following are data collected on the distribution of zolpidem in eight cases presented to the Office of the Chief Medical Examiner over a three-year period.

Experimental

Zolpidem Extraction—To a 5 mL standard, fluid or tissue homogenate were added 2 mL 0.1 N sodium hydroxide, 100 μL of 100 mg/L ethyl morphine (internal standard solution) and 20 mL n-butyl chloride. After mechanical rotation and centrifugation, the n-butyl chloride layer was separated and extracted with 3 mL 1 N sulfuric acid. The acid layer was removed, alkalinized with 0.5 mL ammonium hydroxide and extracted with 5 mL methylene chloride. The methylene chloride was transferred to a conical centrifuge tube and evaporated to dryness at 40°C. The residue was reconstituted in 0.1 mL isopropanol and transferred to an autosampler vial for gas chromatographic analysis. Quantitation was based on the area ratio of analyte to the internal standard in comparison to four fortified standards at concentrations ranging from 0.2 to 4.0 mg/L. Appropriate dilution of specimens with distilled water was performed to ensure quantitation within the limits of the standard curve.

Instrumentation—Zolpidem analysis was performed on a Hewlett Packard 5890 gas chromatograph with a nitrogen-phosphorus detector (GC-NPD) and a Hewlett Packard 7673A automatic sampler. The column used was a cross-linked HP-5 fused silica capillary column (25 m × 0.32 mm inside diameter × 0.17 μm film thickness). Helium was the carrier gas flowing at 1 mL/min. The oven temperature began at 100°C for 1 min, increased at 30°C/min to 200°C, increased at 10°C/min to 260°C, and increased at 20°C/min to 300°C, holding for 8 min. Splitless injection mode was utilized.

Drug confirmation was performed using a Hewlett-Packard 5890 Series 2 gas chromatograph equipped with a 5972 mass selective detector. Similar chromatographic conditions as listed above were used. The mass spectrometer was operated in the scan electron impact mode.

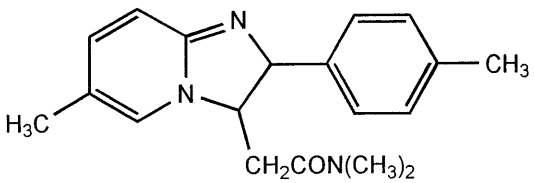

FIG. 1—*Structure of zolpidem.*

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Results

Routine testing for volatiles, therapeutic and abused drugs was performed in all cases. This included volatile testing for methanol, ethanol, acetone and isopropanol by head space gas chromatography, acid/neutral drug testing by GC-NPD, alkaline drug testing by GC-NPD, morphine by radioimmunoassay and acetaminophen, ethchlorvynol and salicylate by colorimetry. Table 1 lists the blood alcohol and drug results in the presented cases. In each case, the presence of each drug was confirmed by full-scan gas chromatography/mass spectrometry. The mass spectrum of zolpidem has a base peak of m/z = 235, with other prominent ions at m/z = 307 (molecular ion) and 219. Table 2 provides the distribution of zolpidem in the postmortem specimens received in each case. Table 3 gives the final disposition of each case.

TABLE 1—*Results of alcohol and drug testing in the presented cases.*

Case	Volatiles, g/dL	Drugs, mg/L*
1	n.d.	diphenhydramine: 0.5 hydromorphone: 0.16 zolpidem: 2.2
2	ethanol 0.02 isopropanol 0.13	pentobarbital: 670 zolpidem: 0.83
3	ethanol 0.02	sertraline: 0.9 desmethylsertraline: 3.7 zolpidem: 0.28
4	n.d.	morphine: 0.75 zolpidem: 0.18
5	n.d.	amitriptyline: 18 fluoxetine: 24 nortriptyline: 21 norfluoxetine: 1.7 zolpidem: 0.22
6	n.d.	zolpidem: 0.58
7	n.d.	diphenhydramine: 9.2 zolpidem: 3.2
8†	n.d.	zolpidem: 0.19 secobarbital: 23

n.d. = None detected.

* All quantitative values reported were measured in heart blood except case 5 where liver results are reported.

† Carboxyhemoglobin saturation value was 3%.

TABLE 2—*Distribution of zolpidem in the presented cases.*

	Concentrations (mg/L or mg/kg)							
Specimen	1	2	3	4	5	6	7	8
Heart blood	2.2	0.83	0.28	0.18	. . .	0.58	3.2	0.19
Periph. blood	2.5	. . .	. . .	4.4	0.12	0.71	3.6	. . .
Bile	. . .	0.20	0.14	0.20	0.08	0.17	. . .	0.07
Urine	1.2	0.09	0.15	0.08	. . .	0.06	0.6	. . .
Liver	2.8	2.5	0.47	0.27	0.22	0.56	3.4	0.16
Kidney	6.6	0.50	0.30	0.34	0.10	0.33	0.95	0.08

TABLE 3—*Final case disposition.*

Case	Cause of Death	Manner of Death
1	drug intoxication	suicide
2	drug intoxication	suicide
3	drowning	undetermined
4	morphine intoxication	undetermined
5	drug intoxication	undetermined
6	multiple injuries	suicide
7	drug intoxication	suicide
8	thermal injuries	undetermined

Discussion

A variety of methods for the identification and quantitation of zolpidem have been published. Liquid chromatography using either ultraviolet of fluorescence detection has been employed (3–6). Multiple gas chromatographic methods for zolpidem quantitation have also been published (7–10). For the presented cases, a routine alkaline extraction followed by a back-extraction into acid and reextraction after alkalinization was performed. A concentrated extract was then injected into the gas chromatograph with a HP-5 column and a nitrogen-phosphorus detector. Under these conditions, no derivatization was necessary. The limit of quantitation was 0.05 mg/L while the upper limit of linearity was 8.0 mg/L. The relative retention time of zolpidem to internal standard was 1.27. Not surprising, the drug elutes from the column in a region close to other benzodiazepines; it elutes after diazepam and chlordiazepoxide, but prior to alprazolam.

The tissue distribution data in Table 2 illustrates multiple points. In six of the eight cases, both blood and urine were analyzed. In each case, the blood concentration was higher than the urine concentration. This is consistent with the extensive metabolism of the drug and indicates that blood would be the preferred specimen for screening as opposed to urine. No attempts were made to identify or quantitate any of the metabolites of zolpidem. The distribution of zolpidem into the liver and kidney failed to identify any sequestration of the drug into either specimen. This is expected, since the volume of distribution of zolpidem is less than 1 L/kg. Since the brain is not routinely collected for toxicologic analysis, no study of the distribution of the drug into the brain was made. Moreover, in the four cases where central and peripheral blood specimens were quantitated, three provided similar results, while the other case (#4) showed widely different concentrations between the specimens. A review of the case failed to account for this difference.

There have been several reports of fatalities involving zolpidem. In one case, death was attributed to hypothermia following zolpidem ingestion; a blood zolpidem concentration of 0.52 mg/L was found in this case (11). Tracqui et al. (12) reported a suicidal intoxication of zolpidem and acepromazine with blood, urine and bile concentrations of zolpidem of 3.29, 2.54 and 1.27 mg/L, respectively. Meeker et al. (13) published a case report of a multiple drug intoxication of zolpidem, hydrocodone and morphine. Zolpidem concentrations were as follows: heart blood: 2.91 mg/L; peripheral blood: 1.40 mg/L; urine: 2.13 mg/L; and liver: 4.74 mg/kg. In addition, 172 mg of zolpidem was found in the gastric contents. A combined ethanol and zolpidem fatality was reported by Khodasevitch and Volgram (14). A blood zolpidem concentration of 0.9 mg/L was combined with a 0.25 g/dL blood ethanol concentration. In this case, the liver concentration was 42 mg/kg and the kidney concentration was 4.8 mg/kg, respectively. Lichtenwalner and Tully (15) reported a blood zolpidem concentration of 7.9 mg/L in an elderly woman who drowned in her bathtub.

Five of the cases presented were deaths due to drug intoxication. In cases 2, 4 and 5, zolpidem was an incidental finding because the drug fatalities resulted from other drugs. In cases 1 and 7, zolpidem was present in elevated concentrations and was a contributing, but not exclusive cause of the drug intoxication. The remaining three cases were deaths that were not caused by drugs. The blood zolpidem concentrations in these cases were therapeutic (0.28, 0.12 and 0.19 mg/L, respectively).

References

1. Salva P, Costa J. Clinical pharmacokinetics and pharmacodynamics of zolpidem, therapeutic implications. Clin Pharmacokinetics 1995; 29:142–53.
2. Langtry H, Benefield P. Zolpidem—A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential. Drugs 1990;40:291–313.
3. Tracqui A, Kintz P, Mangin P. High-performance liquid chromatographic assay with diode-array detection for toxicological screening of zopiclone, zolpidem, suriclone and alpidem in human plasma. J Chromatogr 1993;616:95–103.
4. Ascalone V, Flaminio L, Guinebault P, Thenot JP, Morselli PL. Determination of zolpidem, a new sleep-inducing agent, and its metabolites in biological fluids:pharmacokinetics, drug metabolism and overdosing investigations in humans. J Chromatogr 1992;581: 237–50.
5. Guinebault P, Dubruc C, Hermann P, Thenot JP. High-performance liquid chromatographic determination of zolpidem, a new sleep inducer, in biological fluids with fluorescence detection. J Chromatogr 1986;383:206–11.
6. Debailleul G, Abi Khalil F, Lheureux P. HPLC quantification of zolpidem and prothipendyl in a voluntary intoxication. J Anal Toxicol 1991;15:35–7.
7. Stanke F, Jourdil N, Bessard J, Bessard G. Simultaneous determination of zolpidem and zopiclone in human plasma by gas chromatography-nitrogen-phosphorus detection. J Chromatogr B 1996; 675:43–51.
8. Gaillard Y, Gay-Montchamp J-P, Ollagnier M. Simultaneous screening and quantitation of alpidem, zolpidem, buspirone and benzodiazepines by dual-channel gas chromatography using electron-capture and nitrogen-phosphorus detection after solid-phase extraction. J Chromatogr 1993;622:197–208.
9. Debruyne D, Lacotte J, Hurault de Ligny B, Moulin M. Determination of zolpidem and zopiclone in serum by capillary column gas chromatography. J Pharm Sci 1991;80:71–4.
10. Ahrens B, Schutz H, Seno H, Weiler G. Screening, identification and determination of the two new hypnotics zolpidem and zopiclone. Arzheimittel-Forschung/Drug Res 1994;44:799–802.
11. Augsburger M, Giroud C, Lucchini P, River L. Suicide involving zolpidem and hypothermia. The International Association of Forensic Toxicologists 31st International Meeting Congress on Forensic Toxicology; 16–20 Aug. 1993 Leipzig, Germany, 17–22.
12. Tracqui A, Kintz P, Mangin P. A fatality involving two unusual compounds-zolpidem and acepromazine. Am J Forensic Med Pathol 1993;14:309–12.
13. Meeker J, Som C, Macapagal E, Benson P. Zolpidem tissue concentrations in a multiple drug related death involving Ambien®. J Anal Toxicol 1995;19:531–4.
14. Khodasevitch T, Volgram J. A fatality involving zolpidem. Bulletin of the International Association of Forensic Toxicologists 1996; April 26(2):37–9.
15. Lichtenwalner M, Tully R. Case report: a fatality involving zolpidem. J Anal Toxicol 1997;21:567–9

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