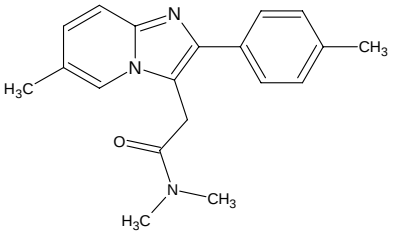
Zolpidem in lethal cases

MARIE STAŇKOVÁ, PETR KURKA

Institute of Forensic Medicine, University Hospital Ostrava,
17. listopadu 1790, 708 52 Ostrava-Poruba, Czech Republic
Corresponding author: marie.stankova@fnspo.cz

INTRODUCTION

Zolpidem (Stilnox, Hypnogen) is an imidazopyridine derivate with a strong sedative effects used as a short acting hypnotic agent. The authors describe six lethal cases with important role of zolpidem.



CASES HISTORY

CASE I
A 41-year-old man fall from the window. He died in a bath where he had been moved and showered from sludge. Before a fall from window he ate up about 30 pills of Hypnogen.
Cause of death was determined as a traumatic-haemorrhagic shock.

CASE II
A 44-year-old man with a history of psychiatric treatment was found by his relatives at home. Autopsy was performed one day after death with nonspecific findings - hyperemia of organs and cerebral oedema.

CASE III
A 77-year-old female was found dead at home. Packings of drugs were found around the body. During autopsy were found severe arteriosclerosis, hypertrophy and signs of heart failure. Drugs were established as a stressor in heart disease.

CASE IV
A 51-year-old man was found dead in bath. Packings of drugs and his letter were found around the body. Cause of death was determined as a drowning.

CASE V
A 65-year-old female with a history of psychiatric treatment was found dead at home.
The autopsy findings were hyperemia of organs and cerebral oedema.

CASE VI

A 55-year-old man with a history of psychiatric treatment was found dead in the forest. The autopsy findings were nonspecific: hyperemia of the lungs, liver, kidney, spleen, cerebral oedema.

TOXICOLOGICAL ANALYSIS

A: Qualitative analysis

For toxicological investigation we used:
- gastric contents, urines, livers, bloods (subclavian artery blood)

Isolation

Urine and gastric contents - were extracted with diethylether after acidification and alkalization.
Liver – 5 g of liver was homogenized and precipitated with ammoniumsulphate method and after pH adjustment (pH 4) SPE extraction was performed. SPE, 6 ml, 400 mg Evidex; condition: 6 ml of methanol, 6 ml of water; load sample; rinse: 6 ml of water, vacuum, 1 ml of n-hexane, vacuum; elute: 2 ml of ethylacetate-methanol (98:2) and 2 x 2 ml of dichlormethane-isopropanol-ammonium hydroxide (4:1:0,1). Combined eluates were evaporated under nitrogen at 40°C and reconstituted with 0,1 ml of methanol.

Analysis

TLC screening was performed on silicagel plates - HPTLC-Fertigplatten Kieselgel 60 and DC-Alufolien Kieselgel 60 (fy Merck).
Mobile phase: ethyl acetate-ethanol-conc. ammonia (36:2:2) with detection: UV light 254 and 365 nm, sulfuric acid in ethanol (1:1), UV light 254 and 365 nm, Dragendorff spray, iod in chloroform.
GCMS screening: Shimadzu GCMS-QP 5050A instrument; Ionization mode (EI 70 eV);
Capillary column - Zebron ZB5; 30 m; 0,32 mm I.D.; 0,25 mm film (fy Phenomenex, USA); injection temp.: 250°C, interface temp.: 280°C; temperature programme: 80°C ramped 20°C/min. to 270°C, hold for 15 min.; carrier gas: Helium at constant flow 2,2 ml/min.; spectrum scanning: 50 - 500 AMU.

B: Zolpidem determination

Extraction: 0,2 ml of blood or serum was alkalized with 0,4 ml of borate buffer (pH 9) and extracted with 1 ml of toluene. Propiverine was added as the internal standard. Organic layer was evaporated under nitrogen at 50 °C and reconstituted with 0,2 ml of mobile phase.
Analysis: Analyses were performed with LC-MS 2010A by Shimadzu with APCI in positive mode. For the separation, an analytical column - Gemini C18, 50 x 2 mm, 5 um (Phenomenex) - was used. Mobile phase was completed 0,01 M ammonium acetate buffer with 0,1 % formic acid(MFA) and acetonitrile (MFB) in a gradient mode. A gradient starting with 15 % MFB was increased linearly to 90 % MFB over 15 minutes, then decreased do 15 % MFB. The flow rate was 0,2 ml/min. m/z for zolpidem - 308, for IST (propiverin) – 368.

RESULTS

LC-MS-APCI method was successfully applied to samples of clinical and forensic cases. The method produced linear response curves (from 2 to > 1000 ng/ml) with correlation coefficient

higher than 0,99. The extraction procedure gives the mean recovery 80 % at 200 ng/ml. Limit of detection (LOD) in blood was about 0,5 ng/ml and limit of quantification (LOQ) was about 2 ng/ml (Table 1). Results of intraday precision of zolpidem determination are in the table 2. Accuracy was verificated using Student´s t-test.

Table 1: Recovery, LOD, LOQ and correlation coefficient of calibration of zolpidem

	Recovery (%)	RSD (%)	LOD (ng/ml)	LOQ (ng/ml)	R2
zolpidem	80	5,8	0,5	2	0,9987

Table 2: Intraday precision results; N = 8

Target (ng/ml)	Mean ± SD (ng/ml)	RSD (%)
50	50,7 ± 1,8	3,6
200	200,1 ± 11	5,6
1000	1034 ± 62	6,0

Figure 1: Calibration curve of zolpidem

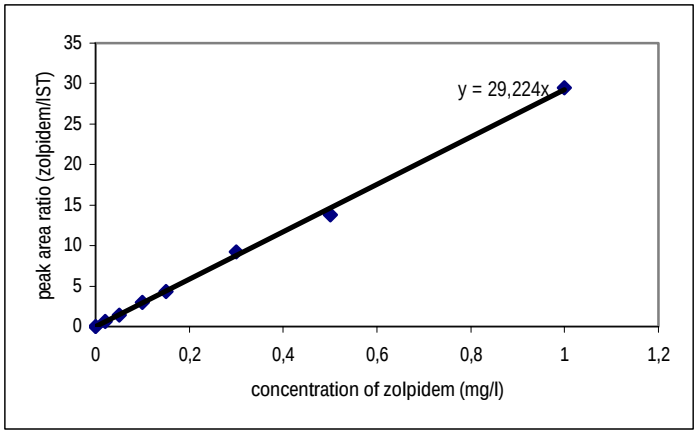
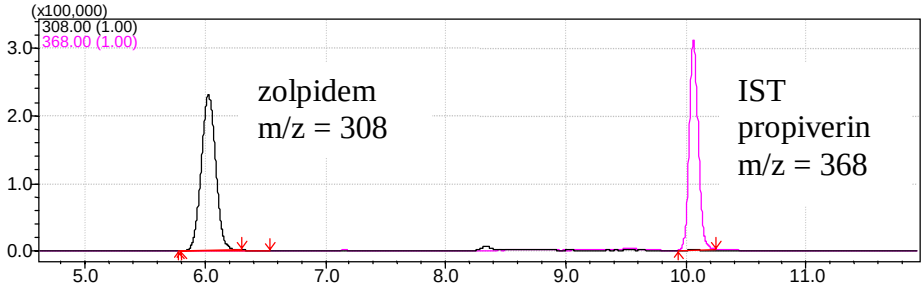


Figure 2: LC-MS analysis of zolpidem with internal standard (propiverin)



CASE RESULTS

case	gender/age	**ZOLPIDEM**	other drugs	blood level
I	M/49	0,60	clomipramine	0,01
II	M/44	1,03	alcohol	2,08*
III	F/77	1,22	metoprolol	0,02
IV	M/51	1,23	betaxolol	2,47
V	F/65	3,02	citalopram	11,40
			betaxolol	5,43
			verapamil	20,15
VI	M/55	1,10	maprotilin	12,20

Blood level: mg/L - drugs; g/kg - alcohol

CONCLUSIONS

Zolpidem as a short acting hypnotic agent is widely used for the treatment of insomnia. Zolpidem in itself is relatively low toxicity. Their toxicity is higher in combination with other CNS active drugs or ethanol. In all six presented cases there are zolpidem blood levels in toxic or lethal range. In three cases (II, V and VI) there cause of death was establish as overdose with medicaments. In one case (IV) the cause of death was suffocation in consequence of drowning, in one case (I) the cause of death was traumatic shock due to fall from window. In one case (III) zolpidem was established as a stressor in heart disease.
Presented LC-MS-APCI method is sensitive and selective for determination of zolpidem in blood. This method is applicable for routine toxicological practise.

Zolpidem - Summary of data

- an imidazopyridine derivate
- a short -acting hypnotic agent for insomnia
- a benzodiazepine-receptor 1 agonist
- after oral administration, the bioavailability of zolpidem is approx. 70 %
- maximum plasma concentrations are reached in 0,5 to 3 hours
- plasma protein binding is approx. 92 %
- $T_{1/2}$ = 2,4 hours (range 0,7 to 3,5 hours)
- elimination as inactive metabolites mainly in the urine (approx. 60 %)

Overdose

- single drug overdose:
  • mild symptoms as are mental confusion and mental lethargy
  • ataxia, hypotonia, hypotension, respiratory depression, rarely coma after high doses of zolpidem single
- combined intoxication with CNS active drugs or ethanol:
  • a comatose state, respiratory depression, possibility death

Zolpidem blood concentrations (mg/L)[1]	-	therapeutic:	0,08 – 0,15 (0,20)
		toxic:	0,5
		lethal:	2 - 4

References:

1. Schulz M., Schmoldt A.: Zusammenstellung therapeutischer und toxischer Plasmakoncentrationsbereiche von Arzneistoffen, Anaesthesist, 43, 835-844 (1994)