Comparative Study of Drug Interactions in Benzodiazepine Associated Deaths

CARMEN LIDIA CHITESCU[1,2], ELENA LACRAMIOARA LISA[2]*, IULIU FULGA[1,2], ELPIDA PALTENEA[2], MONICA MORARU[1,2], COSTINELA VALERICA GEORGESCU[2], IONUT CLAUDIU VASILE[2]

[1]Emergency Hospital of Galati, Department of Legal Medicine,177 Brailei Str., 800578, Galati, Romania

[2]Dunarea de Jos University of Galati, Faculty of Medicine and Pharmacy, 35 A.I. Cuza Str., 800010, Galati, Romania

Due to their widespread use and availability, benzodiazepines are one of the most common classes of drug associated with both unintentional and suicidal drug deaths. The presented work, conducted at the Legal Medicine Service of Galati aims to expound the role of pharmaco-toxicological interactions between benzodiazepines and other psychoactive substances in drug related deaths. A retrospective analysis of 13 cases of fatal substances overdose was done. Parent drugs and metabolites detection and quantification in postmortem specimens were performed by HPLC-DAD, as part of a full forensic autopsy. The substance interactions were investigated and interpreted in the context of the available medical history. The study results emphases the acute toxicity of a given drug /alcohol combination.

Keywords: benzodiazepines, drug interactions, toxicology, HPLC analysis

Benzodiazepines were introduced into therapy in the 1960s for the treatment of insomnia and are still one of the most prescribed psychoactive drugs for treating a variety of symptoms, including anxiety, insomnia, stress-related disorders, epilepsy, muscle spasm [1]. In Romania, the release of psychotropic and narcotic drugs, required a special medical prescription [2].

Benzodiazepine overdoses results in a typical sedative-hypnotic toxidrome which is characterized by depressed level of consciousness, hyporeflexia, respiratory depression, hypotension and bradycardia [3,4].

Although the toxicity of benzodiazepines alone is generally considered mild [5], the risks from over sedation increase when they are combined with other substances with sedating properties [6]. However, due to their widespread use and availability, benzodiazepines are one of the most common classes of drug associated with both unintentional and suicidal drug deaths [6-8].

Postmortem forensic toxicology revealed that overdoses involving prescription drugs in Romania have increase over the past 10 years and benzodiazepine-type drugs are the most common in drug-related mortality [9]. According to the legal Medicine Service of Galati records during the last 5 years, in more than 50% of the medicines related deaths the toxicological analysis revealed the presence of benzodiazepines as: diazepam, midazolam, alprazolam, clonazepam.

The analyses conducted in the study presented below used forensic toxicology database of the Legal Medicine Service of Galati. The present work aims to expound the role of pharmaco-toxicological interactions between benzodiazepines and other psychoactive substances as alcohol, barbiturates, antidepressants, or antiarrhythmic agents in drug related deaths. A retrospective analysis of 13 cases of fatal substances overdose was presented. Parent drugs and metabolites detection and quantification in postmortem specimens were performed as part of a full forensic autopsy. The substance interactions were investigated and interpreted in the context of the available medical history in order to determine whether and how the drugs measured played a role in the cause of death.

The results of the study have implication not only to support a better interpretation of postmortem drug toxicology but for the safety of drug administration in general.

Experimental part

Material and methods

Postmortem forensic pathology reports for autopsies examined at the Legal Medicine Service of Galati between 2013 and 2017 were reviewed for the purpose of this study. All postmortem reports, where the cause of death was attributed or associated to substance overdose were assessed. The occurrence of other medicines or ethanol in benzodiazepines related deaths was investigated. Thirteen cases of death due to combined toxicity of benzodiazepines and other substances interactions were identified.

All data collected as routine laboratory analyses were part of forensic autopsy. Clinical data including diagnostic information or drug prescription, and previous drug abuse in recreational purpose, were available for 9 cases (table 1).

Drugs quantitation was performed on high performance liquid chromatography/ diode-array detection (HPLC/DAD).

Chemicals, reagents and materials

Organic solvents used were purchased from Merck Romania: methanol, acetonitrile, methylene chloride, diethyl ether. Formic acid (98%), phosphoric acid 85%, Tris(hydroxymethyl)aminomethane, acetic acid, amonium acetate, Potassium dihydrogen phosphate, ultrapure water (LC-MS grade) were purchased from Merck Romania. β-Glucuronidase/Arylsulfatase from Helix pomatia were provided by Sigma Aldright (Germany) Analytical standards: diazepam, alprazolam, phenobarbital, codeine, carbamazepine, levomepromazine, lidocaine were purchased form Lipomed GmbH, Germany

Analysed samples

The specimens provided for qualitative and quantitative analysis was: femoral blood, urine, tissue fragments, and

* email: elena.lisa@ugal.ro

Table 1

THE CASES OF DEATH DUE TO COMBINED TOXICITY OF BENZODIAZEPINES AND OTHER SUBSTANCES INTERACTIONS IDENTIFIED BETWEEN 2013-2017 IN THE LEGAL MEDICINE SERVICE OF GALATI

Case nr	Genbder	Age	Case history	Cause of death
1	F	29	Epileptic person Found death at home. Empty blisters of levomepromazine, midazolam and doxepine were found in the victim's room	voluntary poly-drug intoxication
2	F	21	History of abuse of psychoactive substances for recreational purposes. Recently diagnosed with takotsubo cardiopathy Found death in a hotel room. An empty sealed container of "Lidocaine Spray 10%" with its spray was found. A suicidal note by SMS to the family was sent by the victim	voluntary poly-drug intoxication
3	M	22	History of abuse of psychoactive substances for recreational purposes. Brought to the emergency room in coma due to ingestion of alcohol and drugs. Death was recorded after 4 hours.	Accidental poly-drug intoxication
4	M	30	No information available. Found death in his home bathroom	Voluntary drug intoxication
5	M	34	Schizophrenia; under psychiatric treatment	Voluntary poly-drug intoxication
6	M	28	No information available. Found death at home	Mechanical asphyxia by suffocation
7	F	28	Depression; under psychiatric treatment	voluntary poly-drug intoxication
8	M	30	No information available. Found death at home	Accidental drug intoxication
9	F	68	History – depression, hepatitis C	Voluntary poly-drug intoxication
10	M		No information available. Found death at home	Voluntary poly-drug intoxication
11	F	52	Depression; under psychiatric treatment. Empty blisters of carbamazepine, fluvoxamine and nitrazepam were found in the victim's room	Voluntary poly-drug intoxication
12	F		Depression; under psychiatric treatment. Empty blisters of diazepam were found in the victim's room	Voluntary poly-drug intoxication
13	F	62	Depression; under psychiatric treatment. Empty blisters of xanax were found in the victim's room	Accidental poly-drug intoxication

gastric content, obtained during autopsy. In all presented cases, the samples were collected in no more than 48 hour after death. All samples were stored frozen (-20°C) until were analysed.

Sample clean-up and concentration

LL extraction for blood samples: blood samples and tissue homogenate (5 g of tissue / 15 mL ultrapure water) were extracted with methylene chloride (2.5 mL sample / 5 mL solvent) in both basic (*p*H 9, with Tris reagent) and acidic condition (*p*H 3, with acetic acid 4%). After centrifugation, the extract was evaporated under a high purity nitrogen flow at 40°C (Thermo Scietific, Germany). The residue was reconstituted in 500 µL methanol and filtrated thru 0.2 µm micro-filter.

Urine enzymatic hydrolysis followed by LL extraction: a volume of 500 µL of urine was adjusted to *p*H 5.5 with 195 µL acetate buffer and incubated for 2.5 h at 37°C with 7.5 µL β-glucuronidase. The urine was then extracted by liquid-liquid extraction with methylene chloride in basic condition (*p*H 9, with Tris reagent). After evaporation, the residue was dissolved in 250 µl methanol and filtrated (0.2 ∝m).

Gastric content LL extraction: a volume of 5 mL gastric content was filtered and extracted with methylene chloride/ diethyl ether (70/30). After evaporation, the residue was dissolved in 500 µl methanol and filtrated (0.2 µm).

HPLC-DAD analysis

HPLC analyses were performed with an Agilent 1260 Infinity HPLC-DAD system (Agilent Technologies, SUA). For separation a HPLC Column Lichrosphere PR8ec, (Merck/ Darmstadt, Germany) was used. The mobile phase consisted of phosphate buffer *p*H 2.3 (4.6 g KH_2PO_4 / 1 L ultrapure water, *p*H adjustment with phosphoric acid 85%) and acetonitrile (37:63) and had a flow rate of 1.0 mL/ minute. The total run time of the method was 30 min. The detection wavelength was 225 nm, and the full spectra were recorded over a range of 195-380 nm, with a step of 1nm.

Data were evaluated by ChemStation software from Agilent. Analytes identification was based on the comparative analysis of the UV spectrum and the retention parameter (relative retention time) with corresponding data stored in the Pragst spectra library (2007 edition). Confirmatory analysis was done using analytical standards if available or active substance extracted from pharmaceutical formulation in methanol and diluted to appropriate concentration.

The HPLC/DAD system was used to quantitate diazepam with a linear range of 0.5-10 µg/mL, alprazolam with a linear range of 0.1-5µg/mL, lidocaine, levo-mepromazine and fluvoxamine with a linear range of 0.5 - 10 µg/mL, carbamazepine with a linear range of 10- 75 µg/m, phenobarbital with a linear range of 1.5 -20 µg/mL, and doxepine, midazolam and codeine with a linear range of 0.1-2.5 µg/mL. The working standards were serially diluted to comprise a six point calibration curve.

Results and discussions

Out of all autopsy performed in the studied period (2013-2017), the death cause of 53 cases (1.8 %) was substances intoxication. A proportion of 47% (25 cases) of these was medicines intoxication. Pesticides (38% of cases), methanol (6%), ethylene glycol (5%) or caustic substances (4%) were founded. Ethanol fatal intoxications (12 cases representing 0.4 % of all deaths investigated in the Legal Medicine Service Galati during the study period) were not included.

Regarding the substances related death, in 13 (52 %) cases of 25 medicines related intoxication analysis results for benzodiazepines or benzodiazepines metabolites were positive. The suicide prevalence was of 75 % in the target group. Three cases (25%) of accidental overdose of psychoactive substances were recorded. Equal proportion (50%) of man and women victims was observed. The age distribution of fatal intoxications had the highest frequency for the age-group 28-30 years.

The result of the toxicological analysis are summarised in the table 2. In addition to benzodiazepines (diazepam, alprazolam, midazolam and clonazepam), the other substances found included: ethanol, phenobarbital, codeine, neuroleptics (levomepromazine), antiarrhythmic agent propafenone and lidocaine. The combination of benzodiazepine and barbiturates occurred in 5 cases (42%). Due to the lack of analytical standards, no quantification of the metabolites was done using the Pragst Spectra Library (match factor higher than 0.990 and a purity factor of 990% were considered).

In the context of systematic toxicological screening high performance liquid chromatography wit diode array detection (HPLC-DAD) assisted by Pragst comprehensive UV spectra library proved to be a useful tool in substances identification and quantification in biological samples. However, chromatographic techniques are currently used for pharmaceuticals separation and identification [10,11].

Post mortem toxicology

Limitations in this study include the potential role of postmortem redistribution in altering drug concentrations. Post mortem process as autolysis, bacteria and enzymatic activity, body temperature decrease, blood settling in the direction of gravity and rigor mortis can lead in important changes in drug concentration in post mortem samples [12]. Correct interpretation of postmortem drug concentrations is essential in forensic pharmacology, cause of death determining and recent antemortem events establishing. However, the decomposition process makes it practically impossible to apply the pharmacokinetic parameters to deceased people [12, 13].

Femoral blood samples are probably as reliable as samples obtained antemortem being less affected by postmortem redistribution through drug diffusion from gut or other sites of high concentrations [12,14]. Easy to collect, urine samples contain either parent drug or metabolite(s). Collection of gastric contents can give information regarding to very recent ingestion of substances and samples of solid organs as heart, lung, and liver usually contain high drug concentrations.

In the attempt to gain as much information as possible from post-mortem toxicological analyzes, numerous studies have been conducted. Liver/blood concentration ratios were considered for determining the time of death [15]. A liver/blood ratio of >4 indicates death occurred

Table 2

TOXICOLOGICAL ANALYSIS RESULTS

Case nr.	Substances identified	Quantities of substances in analysed samples					Identified metabolites in blood
		Femoral blood $\mu g\ ml^{-1}$	Liver $\mu g\ mg^{-1}$	Kidney $\mu g\ mg^{-1}$	Urine $\mu g\ ml^{-1}$	Gastric content $mg\ g^{-1}$	
1	Levomepromazin	NF	NF	NF	-	identified	Nordoxepin
	Midazolam	0.6	NF	NF	-	identified	
	Doxepin	1.0	3.5	2.5	-	identified	
2	Lidocaine	27	63	30	9	17.5	Nordazepam
	Diazepam	12	1.5	NF	NF	26	
3	Diazepam	3	-	-	-	-	Nordazepam Norcodeine
	Amobarbital	2.5	-	-	-	-	
	Codeine	0.25	-	-	-	-	
4	Propaphenone	1.8	-	-	-	identified	NF
	Diazepam	4.9	-	-	-	identified	
5	Phenobarbital	48	22	-	70	-	Nordazepam
	Diazepam	2.7	3.8	-	1.2	-	
	Paracetamol	3	-	-	-	-	
6	Ethylic alcohol		-	-	-	-	7-amino-clonazepam
	Clonazepam	NF	NF	NF	-	-	
7	Phenobarbital	55	62	-	53	identified	Nordazepam
	Diazepam	1.1	NF	-	NF	identified	
8	Ethylic alcohol	1.5	-	-	-	-	Nordazepam
	Diazepam	12	-	-	-	identified	
9	Ethylic alcohol	2.3	-	-	-	-	7-amino-clonazepam
	Phenobarbital	2.6	4	-	-	-	
	Clonazepam	-	-	-	-	-	
10	Alprazolam	0.4	-	-	-	identified	
11	Carbamazepine	91	32	-	-	-	
	Fluvoxamine	15	9.6	-	-	-	3 – hidroxy-nitrazepam
	Nitrazepam	NF	NF	-	-	-	
12	Diazepam	0.9	2.5	-	NF	-	Nordazepam oxazepam, tenazepam (in urine)
	Levomepromazin	3.7	2.6	-	21	-	
13	Phenobarbital	34			73	Identified	α-hydroxy alprazolam 3-hydroxy methyl-5-methyltriazol-yl chlorobenzo-phenone
	Alprazolam	NF	NF		1	NF	

within 5 h of ingestion [15]. An *F* factor (antemortem concentration = postmortem concentration/F) as a means of identifying drug redistribution was proposed [16]. After a recent extensive study, a post-mortem blood/plasma relationship was calculated for each drug [17].

Regarding the substances detected in the presented cases, for phenobarbital, alprazolam, codeine, carbamazepine, the postmortem blood/therapeutic plasma concentration ratio is 1, suggesting that in a comprehensive postmortem data set, median postmortem concentration is within the established therapeutic range [17]. For doxepin and fluvoxamine, this ratio was 3, for midazolam is 2, and for levomepromazine, 16. This suggests that postmortem concentrations might be somewhat higher than they are antemortem due to postmortem redistribution of drugs. In contrast, the postmortem blood/therapeutic plasma concentration ratio of 0.90 for diazepam and 0.27 for lidocaine suggest that postmortem concentrations are lower than antemortem [17].

Therefore, therapeutic or toxic drug concentrations measured in plasma available in literature (table 3) are reliable as reference intervals for interpretation in post-mortem toxicology for phenobarbital, alprazolam, diazepam, codeine, and carbamazepine. On the other hand, correction should be done for lidocaine, levomepromazine, fluvoxamine and doxepin.

Benzodiazepines pharmacology

The central nervous system (CNS) inhibitory effects of benzodiazepines result from an increased frequency of GABA receptor chloride channels opening in the receptor complex followed by the increase of the conductance of chloride ion across the nerve cell membrane, lower of the potential difference between the interior and the exterior of the cell, and blocking the ability of the cell to conduct nerve impulses [4].

After oral administration >90% of diazepam is absorbed. The protein binding of diazepam is around 98.5% and the bioavailability is about 40-100% of oral administered dose [1]. Long-term therapy with diazepam leads steady state plasma concentrations of diazepam of 100-800 ng/mL [20,21]. Diazepam is primarily metabolized by CYP2C19 and CYP3A4 to the major active metabolite, nordazepam. Both diazepam and nordiazepam are hydroxylated to temazepam and oxazepam catalysed by a CYP3A5. Both metabolites are active and highly water-soluble so tend not to accumulate in the body. Oxazepam conjugates with glucuronic acid and form an inactive metabolite [22]. Elimination half-life is from 20 to 50 h (long acting benzodiazepine). Diazepam is excreted in urine mainly as oxazepam glucuronides, small amounts of nordazepam and as parent drug.

For alprazolam, steady-state plasma levels of 20 ng/mL are usually reached within 7 days therapy [23]. Long therapy lead to plasma concentrations ranging from 25 to 55 ng/mL [24]. Due to tolerance development, plasma levels of 100 to 300 ng/mL were reported [25]. Alprazolam is extensively metabolized by oxidation and conjugation with only 20% of the parent drug appearing unchanged in urine [26]. Metabolism is mediated by CYP3A4 and CYP3A5. The major metabolites are 4- hydroxyalprazolam and αhydroxyalprazolam (both pharmacologically active)α-4 hidroxy-alprazolam, and 3-hydroxy-5-methyltriazolyl chlorobenzophenone (HMTBP). Alprazolam average elimination half-life ($t_{1/2}$) is 11 h, ranging from 6 -16 h (intermediate acting benzodiazepine), [23].

Toxicological interactions - cases reports

Toxicological analysis revealed postmortem levels of diazepam ranging from 12 to 0.9 μg/mL in the peripheral blood, 1.5–3.8 μg/g in the liver, 1.2 μg/mL in urine. Concentrations of alprazolam of 0.4 μg/mL in the peripheral blood, and 1.0 μg/mL in urine were measured. According to some studies, diazepam is stable in blood and tissues, even with putrefaction [27]. On the other hands, postmortem blood/therapeutic plasma concentration ratio for alprazolam and diazepam is about 1, which led to the reliable correlations between measured concentrations and reference intervals for interpretation. Therefore, the benzodiazepines measured blood concentrations can be converted to concentrations associated to the therapeutic range and below the toxic doses reported by specialized literature (table 3) [18].

Nitrobenzodiazepines such as clonazepam and nitrazepam are quickly metabolized due to postmortem bacterial activity, resulting in with only metabolites of the parent drugs being detected [28].

Other benzodiazepine metabolites such as: nordazepam, oxazepam, tenazepam, alpha-hydroxy-alprazolam, 3-hydroxymethyl-5-methyltriazolyl chloro-benzophenone (alprazolam urinary metabolite) were identified in urine samples.

Table 3

REFERENCE CONCENTRATIONS IN PLASMA AVAILABLE IN LITERATURE, ALONG WITH LABORATORY RESULTS, FOR INTERPRETATION OF POSTMORTEM WHOLE BLOOD CONCENTRATIONS [18,19]

Substance	Therapeutic plasma concentration μg ml^{-1}	Toxic plasma concentration μg ml^{-1}	Fatal blood concentration μg ml^{-1}	Measured blood concentration μg ml^{-1}
Alprazolam	0.002–0.07	0.04–0.6	0.13–2.1	0.4
Carbamazepine	1.9–13	10–15	20–73	91
Codeine	0.03 – 0.25	0.5 - 1	1-48	0.25
Diazepam	0.02–4	3–20	5–30	Range 0.9-12 Median value -3 Average value – 5.2
Doxepin	0.009–0.15	0.2–2.1	0.9–150	1.0
Fluvoxamine	0.02–0.5	0.65–1.9	2.2–11	15
Levomepromazine	0.005–0.025	>0.4	>0.5	3.7
Lidocaine	1.5-5	6-7	>10	27
Midazolam	0.03–0.4	0.2–2	2.4–62	0.6
Phenobarbital	1–5	8–24	15–241	Range 2.6-55 Median value – 41 Average value – 35
Propafenone	0.20 - 0.60	2-3	2–7.7	1.8

As benzodiazepine toxicity is considered mild, in most of the presented cases death may be explained by pharmacokinetic interactions and lethal synergistic effect of other co-ingested drugs.

Thereby, a classification of the cases can be done on the benzodiazepine contribution in overdose fatality. Three situations were identified:

a) deaths due to benzodiazepines overdose-case 10 (alprazolam concentration in blood exceed the reported fatal concentration);

b) deaths due to benzodiazepine and alcohol overdose - cases no. 6 and 8;

c) deaths due to combined toxicity of benzodiazepine and other pharmaceuticals (cases 1-5, 7, 9, 11-13).

For the last situation, a distinction between cases arises, according to the benzodiazepines role and contribution in the fatal toxicity mechanism. Thus, in cases no. 5 and 7 the high phenobarbital concentrations measured in blood, liver, and urine exceeding the toxic/fatal level, suggest a minor role of benzodiazepines in the death cause. The cases no 11 and 12 are similar, with high measured concentrations of carbamazepine and fluvoxamine and respectively levomepromazine. For the rest of cases, the interactions of benzodiazepines with others pharmaceuticals were decisive in the fatal toxic effect.

Five of thirteen cases (38.5%) involved combination of benzodiazepine and barbiturates as phenobarbital and amobarbital. Concentrations of phenobarbital ranging from 2.6 to 55 μg/mL in the peripheral blood, and from 53 to 73 μg/mL in urine, were measured.

Pharmacokinetic interactions with benzodiazepines refer to phenobarbital induction of CYP3A4 and stimulate the metabolism of many benzodiazepine drugs with decreasing of their plasma concentration [29]. Pharmacodynamics interaction between barbiturates and benzodiazepines mainly refer to synergistic effect by increasing receptor affinity for benzodiazepines [30]. Barbiturates are highly associated with drug-induced respiratory depression [31]. Toxicological effects regard CNS depression, respiratory depression, cardiovascular instability [31]. Due to the high potential for respiratory depression the pharmacologic synergy of these substances increase the risks of combined overdose. These interactions are highlighted in the presented cases.

Case no. 3 was a young male with a history of psychoactive substances abuse. Amobarbital, diazepam, codeine were identified in all post-mortem samples. Nordiazepam was identified in blood and urine. Norcodeine and morphine (codeine metabolites) were identified in blood. All drugs concentrations in blood were lower than toxic/lethal reported concentrations. The cause of death was attributed to cardio-respiratory arrest explained by the pharmacologic synergy of those substances and the combined overdose. This case also illustrates a *classic* drug interaction between opiates and benzodiazepines. Benzodiazepines interact at GABAA sites and opioids interact primarily at μ-receptors [32]. Respiratory depression is the primary mechanism of opioid overdose fatality [33].

Another case showing the interaction of barbiturates with benzodiazepines is case no. 13, a 62 years old woman with depressive disorder. High concentration of alprazolam and phenobarbital were measured in urine. Two of the alprazolam main metabolites were identified. The negative blood results can be explained first, by the longer presence of parent drug (20% unchanged in urine) and metabolites in urine than their parent compound in the blood, and on the other hand, by phenobarbital induction effect on CYP3A4 leading to the stimulation of the metabolism of alprazolam. Considering the half time of alprazolam of 11-16 h, relatively high urine concentration levels provide realistic benchmark for assessing high dose drug use over about 8-10 h before death. Furthermore, considering long elimination half time of phenobarbital between 53 and 120 h with a mean of 79 h [34], the high phenobarbital concentrations measured in blood and urine, demonstrates an unusual drug tolerance.

Case no. 9 shows a fatal co-ingestion of alcohol, phenobarbital and clonazepam in a 68 old woman with depression history. Relatively high alcohol level (2.3 g‰) was measured by titration method. HPLC analysis relived clonazepam ingestion as 7-aminoclonazepam was identified in the post-mortem blood samples (fig. 1). As outlined above, clonazepam is usually not identified in post mortem samples due to enteric bacteria activity of bioconversion. Therapeutic phenobarbital concentrations were measured in blood and urine. Regarding pharmacokinetics interactions between alcohol and

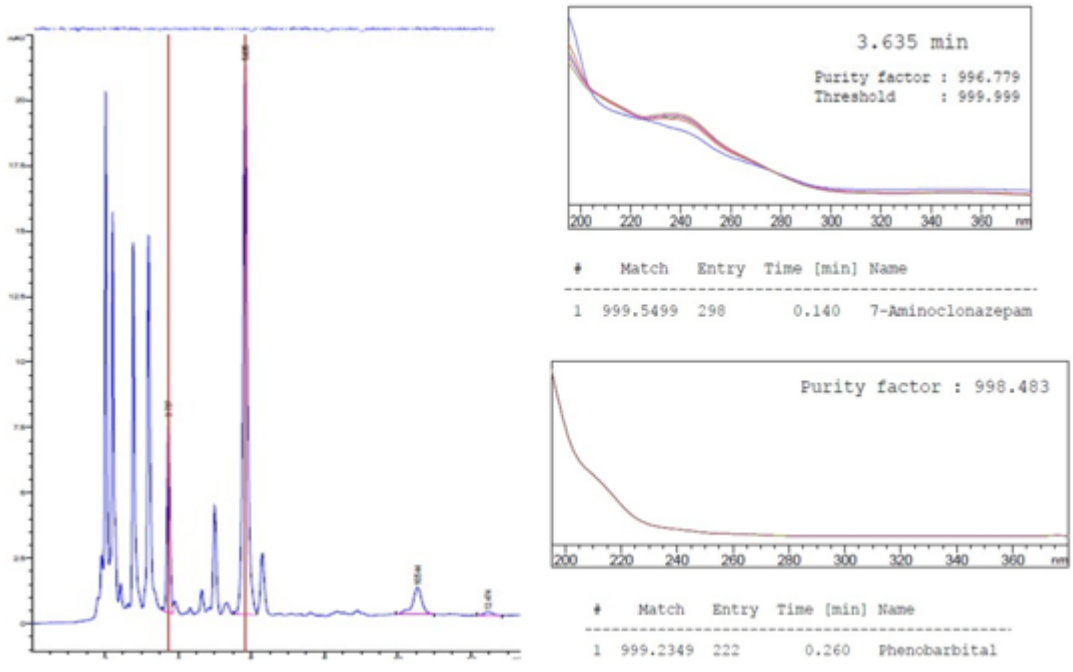

Fig. 1. HPLC-DAD chromatogram of the post-mortem blood sample in case no. 9: 7-aminoclonazepam (RT 3.6 min) and phenobarbital (RT 5.9 min)

benzodiazepines, the phase I metabolism of benzodiazepines becomes competitively inhibited following a significant alcohol intake due the inhibition of the formation of a benzodiazepine–enzyme complex with P450 *via* induction of CYP2E1 [35]. Regarding the toxicological interactions, the combination of high doses of ethanol and benzodiazepines synergistically depress respiration. Concomitant use of benzodiazepines and alcohol with barbiturates is associated with more severe and potentially fatal effects [36].

Except barbiturates, the following substances were identified in the post mortem samples and interact with benzodiazepines leading to fatal toxic effect: lidocaine, midazolam, propafenone, alcohol.

An unusual case of pharmaceutical interaction is represented by the case no. 2, a self-poisoning due to oral ingestion and inhalation of lidocaine aerosol 10% from a spray container in combination with diazepam ingestion. Lidocaine toxicity concerns CNS toxicity and, less common and requiring higher lidocaine plasma levels, cardiovascular toxicity. The symptoms of CNS toxicity including dizziness, sedation, impaired concentration, dysarthria and finally tonic-clonic seizures and coma, becomes increasingly apparent with increasing plasma concentration above 4 µg/mL. Highly effective in the prevention of convulsions [37], diazepam delayed and masked the CNS toxicity symptoms of lidocaine overdose and allows the victim to continue ingestion of lidocaine until toxicity lead to cardiovascular system depression. Diazepam elimination half-life of 24 to 48 h explains the absence of the active substances and its metabolites in the urine sample.

Case no. 1, a 29-year-old female with epilepsy condition, shows the interaction of tricyclic antidepressant with benzodiazepines. Doxepin concentration of 1.0 µg/mL and midazolam concentration of 0,6 µg/mL were measured in blood (fig. 2). Both concentrations reached the toxic level according to the literature. Levomepromazine was found in gastric content but not in blood or urine samples, being probably unabsorbed at the time of death. In humans, doxepin is partly metabolized to nordoxepin. Both substances are pharmacologically active. Nordoxepin was identified in blood samples. Doxepin has substantial anticholinergic and sedative effects [38]. Symptoms of doxepin intoxication are agitation, sinus tachycardia, severe hypotension, seizures and CNS depression, including coma [39]. Death is regularly reported at levels of 2-3 µg / mL^{-1} with most tricyclic antidepressants [19] and can occur either due to the cardiac effect or respiratory arrest [39]. Doxepin may be susceptible to pharmacokinetic interactions when given in combination with inhibitors or inducers of the cytochrome P450 isoenzymes involved in its metabolism: CYP2C19, CYP1A2, CYP2D6 [38]. None of these are involved in midazolam metabolism suggesting less pharmacokinetic interactions. Furthermore, benzodiazepine possesses potent anticonvulsant properties and is usually recommended as a first-line agent to interrupt tricyclic antidepressants-induced seizures and as treatment for status epilepticus [40,41]. In the present case, death seems to have been due to a mixed intoxication with doxepin and midazolam, which may have intensified the central effects of doxepin.

Toxicological analysis performed in the case no 4 - a suicide in a 30 years old male - relieved toxic concentration of propafenone and diazepam (fig. 3). Propafenone is a class 1C (sodium channel blockers) antiarrhythmic drug used for the treatment of ventricular arrhythmias [42]. Propafenone undergoes extensive metabolism *via* the CYP2D6 and CYP3A4. Intoxication with propafenone is rare, a limited number of cases of lethal self-poisoning being reported in the literature [43]. Overdose may induce arrhythmia and conduction disturbance, complex, tachycardia, right bundle branch blocks, congestive heart failure and hypotension, seizures [42]. Interactions of propafenone with diazepam are not documented. In the present case the cause of death has been assigned to the combined toxicity of both medicines.

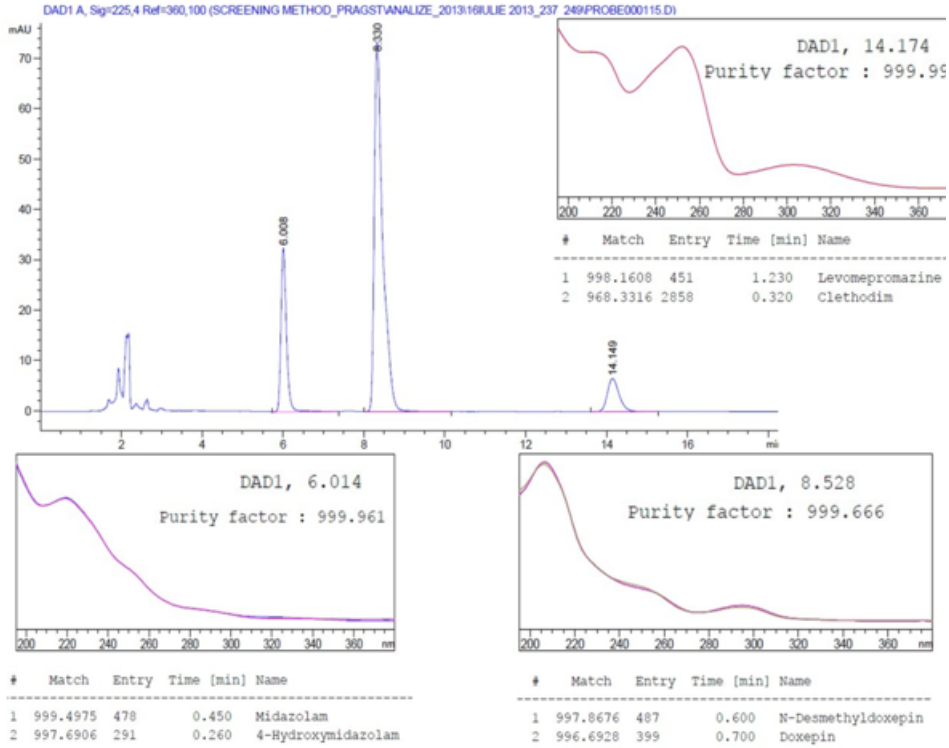

Fig. 2. HPLC-DAD chromatogram of gastric content sample in case no. 1: midazolam, (RT 6.0 min), doxepin (RT 8.5) and levomepromazine (RT 14.17 min)

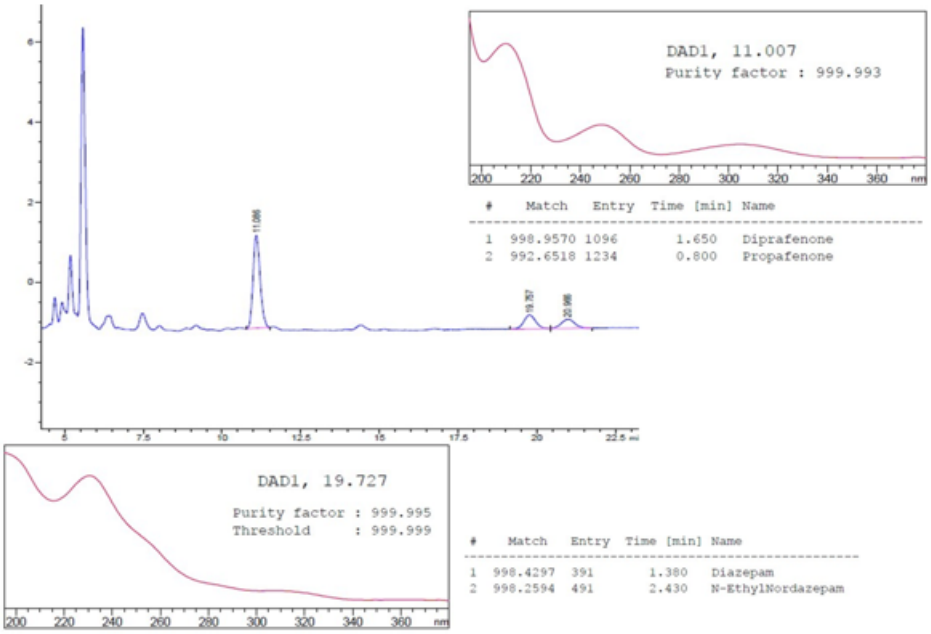

Fig. 3. HPLC-DAD chromatogram of the post-mortem blood sample in case no. 4: propafenone (RT 11.0 min) and diazepam (RT 19.7 min)

Conclusions

Postmortem forensic toxicology frequently deals with combination of drugs related death. Among the drugs most commonly causing fatal poisoning a higher incidence was noticed for the combination of benzodiazepine and other substances as CNS depressants, antiarrhythmic agents, tricyclic antidepressants, opioids, and alcohol.

We describe the toxicological findings in 13 benzodiazepines related fatal intoxications. Analytical data was interpreted with respect of pharmaceutical and toxicological interactions between benzodiazepines and other detected substances. The study results emphases the acute toxicity of a given drug /alcohol combination. CNS depressants have an additive effect, unrelated to the route of benzodiazepine metabolism, on the pharmacodynamics of the benzodiazepines. If an inhibition of metabolism is encountered, synergistic effects may occur.

In conclusion, the forensic pathologist should attribute fatalities not only to the drug perceived as dangerous but consider any drug interaction that can occur. Also, taking into account high prevalence of suicides in fatal poisoning, enhanced vigilance among physicians in observing for abusing patterns of use and suicidal behaviour among patients is required.

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