ESTIMATION OF THE RELATIONSHIP BETWEEN DIAZEPAM USE AND RISK OF VIOLENT DEATH USING *POST-MORTEM* DATA

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Introduction

Modern analytical techniques applied in forensic toxicology give large amount of complex and multidimensional data. In order to extract relevant information. these data can be analyzed using univariate statistical tests or chemometric algorithms for multivariate data analysis. These algorithms are used for exploratory data analysis in order to identify trends/patterns in the data set or to model these trends for classification and prediction purposes.

Objective

Estimation of the possible association between diazepam use and the risk of violent death using chemometric data analysis.

Materials and methods

Samples: Study included data from 293 autopsied cases, during the period 2019-2020.

Method: Benzodiazepine determination included sample preparation using Bond Elut C18 SPE cartridges, derivatization with MTBSTFA and GC/MS analysis on GC 6890N (Agilent, USA).

Statistical analysis:

- Orthogonal projections to latent structures discriminant analysis (OPLS-DA).
- Relative odds ratio (OR) and relative risk (RR). OR and RR greater than 1 were considered statistically significant.

Classification according the cause of death: Group of natural (used as control) and a group of violent death, further divided in group of suicides, accidents and homicides (however, homicides were excluded from evaluation due to the low number of cases).

Classification according to total diazepam concentration:

Concentration level	*Diazepam* (ng/mL)*
I	10 – 100
II	100 – 300
III	300 – 1000
IV	≥ 1000

*total diazepam concentration (including its metabolites)

Results and discussion

- Benzodiazepines were more often detected in the cases of violent death group, mostly detected in the suicide cases (45%). Distribution is shown in Figure 1.
- The OPLS-DA models for comparison of groups of natural death and accidents (Figure 2) and groups of natural death and suicides (Figure 3) revealed good separation between tested groups based on the variables age (followed by temazepam) and temazepam, respectavely.
- Statistically significant RR for the association between total diazepam concentration and the risk of committing suicide was found for diazepam concentration starting at 300 ng/mL (Table 1). Additionally, concentration-dependent risk for committing suicide was also observed.
- The risk for fatal outcome in accidents was statistically significant for total diazepam concentrations > 1000 ng/mL. However, when compared with fatal accidents due to alcohol use, RR of diazepam concentrations in the range of 100-300 ng/mL is similar with RR of alcohol in concentration range 0.5-0.8 g/L.

Conclusion

- The data analyses have shown that the use of diazepam is often associated with violent death.
- The OPLS-DA models comparing natural death cases and accident cases as well as those of natural death cases and suicides showed satisfactory classification performance.
- The identification of temazepam as a metabolite which differs between the groups of natural death and both accidents and suicides is an important finding which can give aid in interpretation of diazepam adverse effects.
- The present study identified relatively strong dose dependent association between the presence of total diazepam above 300 ng/mL and the risk of suicide.

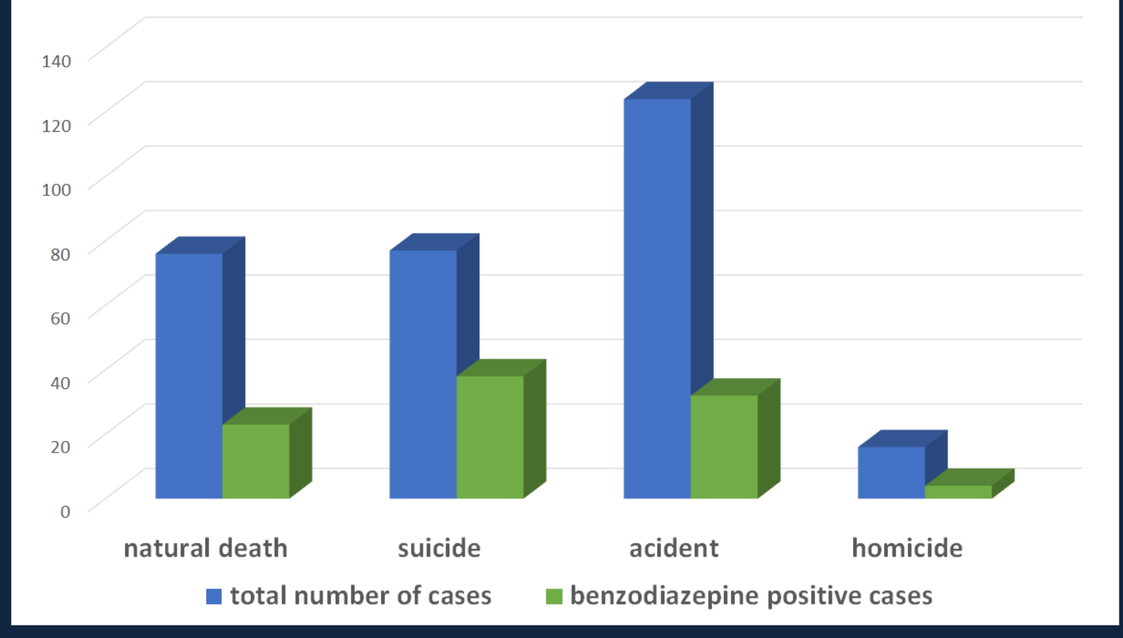

Figure 1. Distribution of death cases.

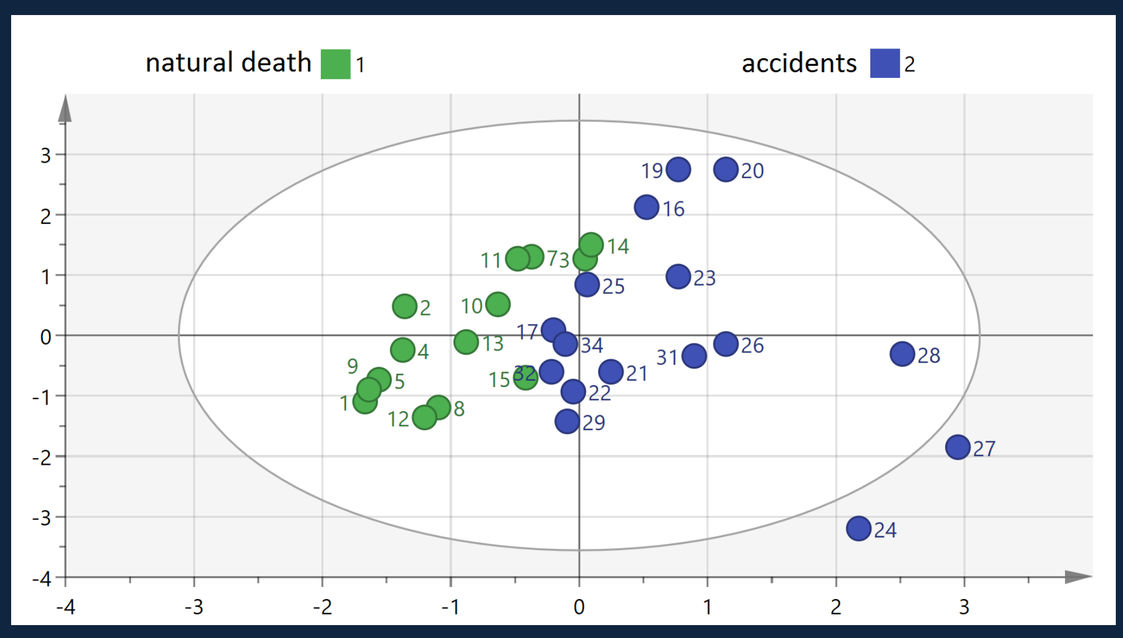

Figure 2. OPLS-DA model (natural death *vs.* accident)

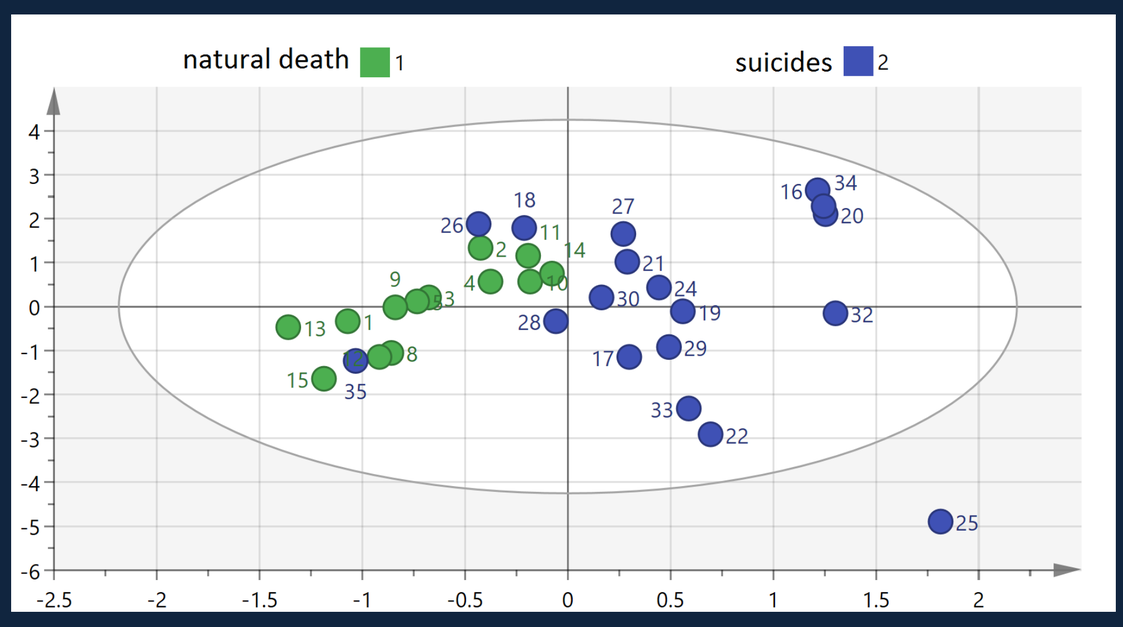

Figure 3. OPLS-DA model (natural death *vs.* suicide)

Conc. level	Suicides		Accidents	
	OR	RR	OR	RR
I	0,74	0,83	0,63	0,82
II	0,25	0,36	0,81	0,92
III	1,59	1,27	0,58	0,79
IV	∞	2,36	∞	1,58

Table 1. Calculated OR and RR for the association between total diazepam concentration and the risk of suicides and accidents

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