„Innovative approach to ante-mortem distribution and post-mortem redistribution of psychoactive substances in human tissues - from animal model to human case samples”

The issue of ***ante-mortem*** (before death) **distribution and** ***post-mortem*** (after death) **redistribution** of psychoactive substances in tissues and vital organs is of great importance especially when classical matrices such as blood are unavailable and alternative matrices e.g. bone marrow aspirate, vitreous humor, liver, have to be studied.

The aim of the project is to determine the **correlation of drugs levels** between tissues, obtain the **concentration-time drugs profiles** and their dependence on **the time since death.**

Psychoactive substances commonly encountered in toxicological practice were selected for the analysis and are belonging to the group of opiates, opioids, amphetamines, benzodiazepines, selective serotonin reuptake inhibitors, and others. Two groups of methods will be utilized throughout the project: **widely used and precise** liquid chromatography coupled with mass spectrometry (LC-MS) method, which will be used with two different sample preparation approaches - Direct Solid Phase Microextraction (in-vivo-DI-SPME) and Dried Blood Spot (DBS), and **minimally destructive** Raman, Infrared, and fluorescence spectroscopy methods.

During the project, the following tasks will be realized. **Task 1** will include the development of **direct in-vivo measurement** of animal and human tissues and vital organs. This step is essential to effectively perform the experiments. The analysis of psychotropic substances distribution will be conducted using DI-SPME sample preparation method. Then, the samples will be analyzed by LC-MS. The appropriate calibration model will be selected. In **Task 2** the development of a **microsampling and microinvasive method for the storage and protection** of biological liquid samples will be realized. This goal of the project is particularly important in cases where the available amount of biological material is small and classical analysis is challenging. In this step, the analysis will be conducted with the use of DBS method and further LC-MS measurements and data analysis carried out in a manner similar to Task 1. The subject of **Task 3** will be the **comparative analysis of psychotropic substances levels** in tissues and vital organs. The concentration of drugs and their metabolites in different matrices will be determined using optimized methodologies developed in Task 1 and Task 2 and compared according to the initial drug doses, time after and ways of administration and time since death. Significance of differences and correlation between the respective biological matrices will be tested statistically. **Task 4** will include the development of **pharmacokinetic models** that describe the concentration-time profiles of individual drugs in tissues. Models will be built based on psychoactive substances concentrations determined in Task 3 in respective matrices. An animal experiment will be used to create models and validate them, so there will be no need for hard-to-reach human dissection material at this stage. Ultimately, the models will be tested on **human case samples in preparation for their use in toxicological practice**. The samples will originate from real autopsy cases with a known medical history and the drugs examined will depend on the circumstances of each case. Finally, in **Task 5**, **sample identification and determination of changes** that occur in vitreous humor and blood samples over time will be conducted with the use of **non-destructive** vibrational and fluorescence spectroscopy methods. Application of this simple spectroscopic methodology will be tested for ante-mortem and post-mortem changes that occur in rabbits tissues. In the next step, human case samples will be studied. Regression models will be built for the determination of the time since death based on the changes observed. In a further step, this spectroscopic approach will be studied for possible drugs determination in biological matrices. Data obtained will be correlated with results of drugs distribution from Task 3.

The implementation of the project will be of great importance to **forensic chemistry and toxicology**. **Animal experiment** will help to expand and systematize knowledge about ante-mortem and post-mortem processes and develop appropriate distribution models of selected psychoactive drugs that can be related to changes occurring **in the human body**.

We strongly believe that our methodology will allow us to gain new knowledge about the ante-mortem and post-mortem changes, which will benefit the society, **as well as contribute to the development of analytical chemistry.**