"Modern techniques for the analysis of biological samples in toxicological and forensic applications"

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Biological samples are common types of evidence and therefore a frequent subject of toxicological and forensic examinations. They can provide a lot of important information on the course of a given event. Proper reasoning requires the development of reliable, effective, but also quick, and easily accessible methods of extracting information from the data recorded during the analysis of biological matrices.

The following techniques were tested in this work:

- fluorescence and Raman spectroscopy to detect biochemical changes in the composition of menstrual bloodstains (MB) in the time since their deposition (TSD) and to develop a TSD estimation model,
- attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and liquid chromatography with a mass detector (LC-MS) to monitor changes in the biochemical matrix composition and the concentration of xenobiotics in biological samples stored under different conditions before the analysis,
- ATR-FTIR spectroscopy, FTIR transmission imaging, and Raman spectroscopy to detect biochemical changes in post-mortem tissues and develop a post-mortem interval (PMI) estimation model.

In the course of the study, it was shown that exposure of menstrual bloodstains to the external environment leads to changes in the content of endogenous fluorophores, tryptophan, NADH, and flavins, which were monitored by fluorescence spectroscopy, and changes in hemoglobin derivatives, which were detected by Raman spectroscopy with a 785-nm laser. Due to the type of markers indicated for biochemical changes, fluorescence spectroscopy showed the potential to develop a universal method for dating stains

of biological traces, while Raman spectroscopy was a method specific for bloodstains with a dominant share of hemoglobin in the spectra. In addition, fluorescence spectroscopy proved to be a sensitive method for estimating early PMI, showing strong changes at the first time points after bloodstain deposition. However, the gradual plateauing of the kinetic curves may indicate that this technique cannot be used to detect TSD changes over 24 hours. Raman spectroscopy was shown to be effective for longer periods of time after deposition. This technique, thanks to the use of imaging and a large amount of registered spectral data, allowed the development of effective models of PLS-DA classification and PLSR regression to date menstrual bloodstains up to two weeks after their deposition. The very good results of the evaluation of the developed models indicated a great potential for their use in forensic practice.

The biochemical processes that occur during the storage of two biological matrices, vitreous humor (VH) and liver homogenate (LH), under three temperature conditions: -20, 4 and 20 °C, were identified primarily as the effect of autolysis decomposition and the action of putrefactive bacteria. The described changes were monitored using ATR-FTIR spectroscopy. Bands at 1590 cm^{-1} for VH and 1404 cm^{-1} for LH, assigned to amino acids, were proposed as potential markers of biochemical changes in samples. Monitoring changes in the intensity of these bands can allow for the assessment of the degree of sample degradation. It should be noted that none of the temperature conditions used allowed for the complete inhibition of changes in the composition of the tested biological matrices; therefore, to ensure their effective storage, it is necessary to use even lower temperatures. To monitor the stability of forensically relevant psychotropic substances in the two tested matrices and the three tested storage conditions, a sensitive LC-MS method with solid-phase microextraction was used. It was found that cocaine, nordiazepam, and venlafaxine, whose concentrations changed significantly during storage under all tested conditions, require particular attention when analysing biological samples stored for some time prior to analysis. The vitreous humor matrix was also shown to provide greater stability of the concentrations of the tested xenobiotics than the liver homogenate matrix.

Raman spectroscopy with UV lasers (199 and 239 nm) turned out to be the best method of identification and proper classification of post-mortem changes in liver samples for the three PMIs studied - 0, 12 and 24 hours. In particular, the use of a 199-nm laser allowed the development of a PLS-DA classification model with 80% efficiency, which indicates great

potential for its practical application. The direction of biochemical changes monitored with the use of this laser was attributed to the degradation of proteins present in the samples. The use of the other two spectroscopic methods tested for this application, ATR-FTIR spectroscopy and FTIR transmission imaging, also showed some advantages. The imaging results allowed to infer the distribution of the lesions in the samples and state that they start from the edge of the tissue. On the other hand, the ATR-FTIR technique, due to the simplicity of sample preparation and measurement, allowed for quick examination of various tissues and body fluids, indicating a high potential for correlating changes in the vitreous humor with PMI. In the context of the mechanism of post-mortem changes, all three spectroscopic techniques used were based on monitoring changes in the content of biomolecules as a result of two main biochemical processes, i.e. decomposition by autolysis and synthesis of new substances as a result of the activity of putrefactive bacteria.

As a result of the conducted studies, it was shown that modern, fast, and significantly shortening sample preparation steps, spectroscopic techniques can be successfully used to identify biochemical changes within the biological samples analysed for toxicological and forensic purposes. Analysis of changes in the content of xenobiotics in the biological matrix was successfully conducted using liquid chromatography with a sensitive mass detector and a modern and environmentally friendly solid-phase microextraction technique.