

According to the World Health Organization (WHO), over 3 million people all over the world die every year due to alcohol abuse. This shows that despite the society's growing awareness of the consequences of excessive alcohol consumption, the problem remains unsolved. However, the most frightening is the occurrence of alcohol consumption by pregnant women because it affects both the health of a future mother and a fetus. The scale of this phenomenon is very big, because every tenth woman in Poland admits to consuming alcohol while pregnant. Any dose of consumed alcohol during pregnancy can cause miscarriage, preterm birth, placental pathology, intrauterine growth restriction, and the occurrence of numerous birth defects. Alcohol has a particularly adverse effect on the structure of the central nervous system, leading to postnatal developmental delays, behavioral disorders, and interferes with the development of craniofacial structures. A group of symptoms caused by toxic effects of alcohol on the fetus are referred to as Fetal Alcohol Spectrum Disorders – FASD. Due to the inadequate state of knowledge in the field of medical diagnostics there are no objective criteria for confirmation of FASD in newborns. Current methods for diagnostic of FASD in newborns are based on noticing certain dysmorphic features, congenital malformations, and inquiring about alcohol consumption during pregnancy while obtaining mother's medical history. However, it is very difficult to obtain such information because many women do not specify the quantities of alcoholic beverages consumed as well as the fact that many different birth defects are characterized by similar symptoms. Therefore, without evidence of alcohol consumption during pregnancy, the diagnosis of FASD is impossible. Ergo, new methods for this purpose are required.

Current studies are focused on the identification of specific compounds (alcohol biomarkers) in neonatal meconium, which presence may indicate prenatal exposure to alcohol. Meconium begins to form at 12<sup>th</sup>-13<sup>th</sup> weeks of gestation, which gives wide time window for the detection of biomarkers. There have been detected fatty acid ethyl esters (FAEE), ethyl glucuronide (EtG) and ethyl sulfate (EtS) as the alcohol metabolites that can potentially be used as biomarkers of alcohol consumption in this biological material. However, the results obtained by different researchers are unclear and cut-off values have not been established yet. This indicates that there is both a need to complement and standardise the current state of knowledge and the development of new analytical methods, which in a quick, cheap and unambiguous way allow to determine alcohol biomarkers in meconium what in turn could confirm alcohol consumption during pregnancy. This opens up a wide area of study and interdisciplinary scientific research.

The determination of biomarkers in meconium, which is characterized by complex composition of the matrix, is very difficult due to: different chemical nature of biomarkers, their low concentration, and the presence of other compounds in the matrix with very similar properties and at the same, or even higher, concentration levels. Thus, it is very important to choose the appropriate methods for sample preparation and techniques used to final determination, which is a big research challenge.

The aim of this project is to develop, select the optimal parameters and to validate a new one or modify already existing analytical methods enabling a determination of FAEE, EtG and EtS in meconium. SPE and new "green methods of extraction", SPME and SBSE, as sample preparation techniques, and GC-MS (the determination of FAEE) and LC-MS/MS (the determination of EtG and EtS) for final determination will be used. To compare developed analytical procedures and the obtained results, statistical and chemometric analysis will be introduced.

The results of this study could contribute to extending current state of knowledge in the context of presence and concentration levels of alcohol biomarkers (FAEE, EtG and EtS) in meconium. Moreover, the results of research could help to estimate cut-off values indicating prenatal exposure to alcohol. Presumably it would allow to confirm alcohol consumption during pregnancy, more explicitly than obtaining mother's medical history. Furthermore, the results will also be used to disseminate the knowledge on the dangers of drinking alcohol during pregnancy, which can significantly reduce the number of pregnant women consuming alcohol, this way reducing the incidences of FASD.

The novelty of the planned scientific research is to develop new analytical methods for the determination of selected alcohol biomarkers in meconium. Additional information will be obtained through the application of chemometric tests to develop multi-parameter measurement data sets. This allows to estimate proof correlation, or its absence, between alcohol biomarkers. An innovative estimation of a correlation of obtained results to: dry weight meconium, birth weight, and Apgar score points, may allow to accurately define the cut-off values of alcohol biomarkers indicating prenatal alcohol exposure.