

A crucial element defining the effectiveness of pharmacotherapy is drug metabolism. Liver, both in humans and animals, is the organ most heavily involved in drug. Effects of the first pass effect can be positive or negative. Some medicines are administered to patients as prodrugs, which must be activated by enzymes located in the liver to demonstrate the pharmacological effect. Hepatic metabolism is also involved in drug-drug interactions. Xenobiotics, including drugs, may cause an increase or decrease in liver enzymes activity which can affect the effectiveness of pharmacotherapy.

Determining the routes of metabolism, the ability to activate or deactivate enzymes in the liver is an extremely important element in the long process of designing new drugs. The use of laboratory animals solely in new drugs candidate metabolism evaluation studies is not possible, because it would involve a high number of such individuals. Therefore, it is an extremely important element in metabolic studies to generate *in vitro* metabolism platform. There are several assays imitating the liver metabolism, including: the cytochrome P450 isoenzymes, liver S9 fractions microsomal enzymes, both fresh and immortalized cells hepatocytes. Creating a system that reflects *in vivo* metabolism pathway as closely as possible will lead to reducing the use of laboratory animals for research. Unfortunately, in each of the systems, the main component is derived from biological systems, which always show bath-to-bath or manufacturer difference. In order to obtain reliable results there is a need to improve the performance of *in vitro* assays. One of the novel methods, which seem to be a promising tool for this purpose, is solid phase microextraction (SPME).

Created in 1990, SPME method enables more precise analysis of selected chemical compounds located in the particular matrix. One application of this method is targeted metabolomics and accurate analysis of chemical compounds, including drugs and their metabolites. The principle of the method is based on the extraction of compounds from the matrix to the surface of fiber or blade coated with a layer of absorbent or adsorbent. In this method, the matrix may be an aqueous solution, e.g. cell culture media or even solid tissue. Simultaneously with extraction process occurs cleaning of the sample and immediate enzymes activity reduction. This process is also called metabolism quenching and is very important in metabolism assessment.

SPME method can be easily carried out in the format of high-throughput analysis of 96-well systems. In this project, we are focused on adaptation and validation of the methodology based on solid phase microextraction to analyze *in vitro* metabolism of drug or drug candidates. Taking advantage from nonselective extraction we can look at all metabolites of the potential pharmaceutical and determine the kinetics of such reaction. These actions can be beneficial in reducing the number of animals used in laboratory studies during preclinical phase of drug development.