ABSTRACT

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Biotransformation enzymes are involved in the metabolism of a wide range of both endogenous compounds and xenobiotics. Regarding the metabolism of xenobiotics, many of those enzymes have not been characterized at all or only marginally. To get better knowledge about how the biotransformation enzymes affect compounds with significant impact on the organism as a whole, they need to be purified, identified and subsequently thoroughly characterized.

The classical electrophoresis is still irreplaceable for protein purification and characterization. Moreover, combination of electrophoresis with mass spectrometry (MS) represents an inseparable tandem for protein identification. However, to obtain unambiguous outputs from mass-spectrometry based protein identification, it is essential to avoid unwanted contamination of the sample during sample preparation.

Wearing disposable protective equipments, using MS grade chemicals, solutions filtering are examples of rules for sample preparation for MS analysis that were established at the Department of Biochemical Sciences. Data obtained from MS analysis showed that as long as these rules are followed, whole sample preparation procedure can be carried out without risk of unwanted contamination at the department. These rules as well as an optimized sample preparation procedure were subsequently applied for the identification of AKR1C3 enzyme obtained by affinity purification from human hepatocytes.