ABSTRACT

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This study was focused on implementation of experimental techniques for purification of human liver microsomal fraction. There were chosen three basic steps of purification process. As a first step, ion-exchange chromatography was chosen followed by two purification steps on the base of gel filtration. Before purification the microsomal fraction had to be solubilized to release proteins from membrane bounds and conditions had to be optimized. Single fractions were subsequently incubated with specific substrate of reductases oracin and the quantity of metabolite 11-dihydroracin was assessed by achiral HPLC analysis. For the most active fractions the SDS-PAGE analysis was done to set size of unknown enzyme. By use of antibody against 11beta-HSD1 was axcleded the presence of this reductase in all analyses fractions. By use of described purification techniques we were successful to concentrate fraction with unknown human liver reductase.