# ABSTRACT

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Title of diploma thesis: HPLC method for determination of testesterone and its use for chatacterization of carbonyl reductases

Prostate cancer belong to hormone-dependent tumors, the proliferation of transformed cells is dependent on activation of the androgen receptor by testosterone. Androgen deprivatin therapy is complicated by productin of active androgens directly in prostate cells. It was proved that among other enzymes also carbonyl-reducing enzymes participate in androgen production, especially enzyme aldo-keto reductase 1C3 (AKR1C3). The aim was to develop a HPLC method for the determination of testosterone and use this method for *in vitro* screening of activity of carbonyl-reducing enzymes in formation of potent androgen testosterone from its inactive precursor androstenedione.

Enzyme samples were incubated with androstenedione c = 20-120 μmol/l and after incubation were extracted by ethyl acetate. Testosterone formed during the incubation was determined by HPLC analysis (column BDS Hypersil $C_{18}$, 5μm, 250×4.6 mm, mobile phase metanol:voda, 70:30, detection: DAD při 240 nm). Samples of human liver subcelular fractions, recombinantly prepared purified cytosolic enzymes and membrane-bound enzymes in the form of Sf9 microsomal fractions with overexpressed target enzyme.

Reducing activity of cytosol, microsomes and also in a number of enzymes (AKR1C1-AKR1C4, AKR1B1, AKR1B10, carbonyl reductase 1, retinol dehydrogenase 4, 17β-hydroxysteroid dehyrdrogenase 7) toward androstendione was proved. kinetic parameters for androstenedione reduction were determined for subcellular fractiona and AKR enzymes. The highest $V_{max}$ expressed as specific activity for AKR1C3, lowest for AKR1C4 was measured. The highest affinity to the substrate was determined for AKR1C3, lowest for AKR1B1.