ABSTRACT

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Title of Diploma thesis: **UHPLC-MS/MS absolute quantification of cytochrome P450 enzymes in C3A, CACO2 modified cell lines and in human liver microsomes**

Cytochrome P450 (CYP) enzymes play a crucial role in drug metabolism. They can be responsible for the failure of treatment, adverse and toxic effects or drug-drug interactions. Modified C3A and CACO2 cell lines with constitutive androstane receptor (CAR) and pregnane X receptor (PXR) might be used for *in vitro* biotransformation and absorption studies instead of primary cell lines. CYP enzymes should be expressed continuously in these modified cell lines. This study aimed to establish the quantitative method for the analysis of the most common CYP enzymes in human: CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2E1, CYP3A5 and CYP3A4 in the above mentioned cell lines and in human liver microsomes. Absolute quantifications of CYP enzymes were carried out by UHPLC in line coupled with tandem mass spectrometry working in scheduled MRM mode. Data assessment was conducted by Skyline 2.6 software. CYP enzymes were not detected in CACO2 and C3A modified cell lines. However, these enzymes were found in human liver microsomes. Average values were ranging from 0.6 pmol/mg to 21.5 pmol/mg of microsomal protein. The lowest detected amounts of CYP protein were 0.006 – 0.210 pmol/mg of microsomal protein in a hundred times diluted human liver sample. These findings point out that CYPs protein levels in modified C3A and CACO2 cell lines were apparently below the limit of detection.

Key words: Cytochrome P450, CACO2 and C3A modified cell lines, Human liver microsomes, Heavy labelled peptide standards, UHPLC-MS/MS, MRM, Skyline 2.6 software