ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové
Department of Pharmaceutical Chemistry and Pharmaceutical Analysis
Candidate: Bc. Martin Smolík
Supervisor: PharmDr. Lukáš Lochman, Ph.D.
Consultant: prof. PharmDr. Petr Pávek, Ph.D.
Title of thesis: Development of LC-MS method for compounds used for determination of cytochrome activity.

Cytochromes P450 (CYP450) are enzymes that play a key role in the metabolism of xenobiotics and some endobiotics (cholesterol, steroid hormones, and eicosanoids). They are present in the liver, gastrointestinal tract, lungs, and kidneys. Determination of CYP450 activity is a valuable tool to detect drug interactions resulting from inhibition or induction of various isoforms of this enzyme.

This thesis is devoted to the development and validation of a new analytical method using liquid chromatography coupled with tandem mass spectrometry for the determination of activity CYP450 isoforms 3A4, 2C9, and 2B6, which are crucial for the metabolism of many drugs and biologically active substances. In the framework of this thesis, a new method was developed providing a fast, accurate, and reliable measurement of the activity of these enzymes based on the quantification of specific CYP450 substrates, namely midazolam (3A4), diclofenac (2C9), bupropion (2B6) and their appropriate hydroxylated metabolites in cell culture medium.

Analytes were separated on a Luna® Phenyl-Hexyl column (3 µm, 100×3 mm, Phenomenex), using a mobile phase gradient. Used mobile phase was composed of 0.05% acetic acid in water and 0.05% acetic acid in acetonitrile (*v/v*). Detection was done using tandem mass spectrometry in selected reaction monitoring (MRM) mode with electrospray ionization. Lower limits of quantification were set at 0.05 µmol/l for bupropion, midazolam, 4-hydroxymidazolam and diclofenac and 0.025 µmol/l for hydroxybupropion, 1-hydroxymidazolam and 4-hydroxydiclofenac.

The newly introduced method was partially validated, where the validated criteria were calibration curves, quality control, accuracy, precision, sensitivity, selectivity, and carry-over.

The validation results showed that the method meets all the acceptance criteria set by the US Food and Drug Administration.

At the end of the diploma thesis, the method's applicability was demonstrated by the analysis of sixteen real samples in a cell culture medium to evaluate the effect of rifampicin on CYP450 in an *in vitro* model of human hepatic cells arranged in 3D. The measured data will become part of the publication with an impact factor.

Keywords:

Analysis, mass spectrometry, liquid chromatography, cytochrome P450, 3D human hepatic cells, midazolam, diclofenac, bupropion, 1-hydroxymidazolam, 4-hydroxymidazolam, 4-hydroxydiclofenac, hydroxybupropion