Abstract

Charles University, Faculty of Pharmacy in Hradec Králové
Department: Department of Analytical Chemistry
Candidate: Dominika Dubecová
Supervisor: PharmDr. Kateřina Plachká, Ph.D.
Consultant: Mgr. Taťána Gazárková
Title of the diploma thesis: Development and validation of SLE method for plasma retreatment prior UHPLC-MS/MS analysis for the determination of steroids

Accurate and reliable determination of endogenous steroid concentrations is an essential part of research into many biological questions. The aim of this work was to develop a sample preparation method that will be subsequently used for the analysis of mouse plasma samples to monitor the effect of acute and chronic stress on the plasma concentration of selected steroid hormones. The set of analysed steroids was compiled based on the requirements of the Institute of Physiology of the Academy of Sciences of the Czech Republic; which carried out the biological experiments.

This diploma thesis focused on the development and optimalization of a sample preparation method for the analysis of 38 steroid hormones; which were subsequently analysed using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Two sample preparation methods were compared within this thesis: protein precipitation (PP) and supported liquid extraction (SLE). The PP method was tested alone; in conjunction with enzymatic hydrolysis and using phosphate buffer solution to optimise the PP method. The composition of the precipitation reagent was also optimized; with the highest recovery achieved using acetonitrile. The highest recovery among the mentioned methods was obtained with PP achieving values of >50% s RSD <15% for most analytes. However; this method was burdened by significant matrix effects; therefore the PP method was further compared with the SLE method.

Several parameters were optimized during the development of SLE method including sample load volume; extraction solvent composition and its volume; and the number of extraction steps. The optimized composition of the loaded sample included 40 μL of mouse plasma diluted by 40 μL of 50% 2-propanol and 320 μL of water. A combination of methyl-tert-butyl ether and ethyl acetate in a ratio of 80:20 (v/v) was selected as extraction solvent. The optimized SLE method enabled to obtain the recovery of > 60% with RSD <15% for majority of the targeted steroids. SLE showed several advantages over PP; including higher recovery and repeatability. The final method was validated according to the ICH M10/EMA guideline. The method was proven to be suitable for fast and precise quantification of plasmatic steroids and can be used for analysis of its stress-dependent changes.

Key words: protein precipitation, supported liquid extraction, mass spectrometry, ultra-high-performance liquid chromatography, optimalization, validation