Abstract

This diploma thesis was focused on the development of a separation method for qualitative analysis of twelve chosen pterin derivates by hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry detection. Two silica gel-based columns with different ligands attached were used for the chromatographic separation – a ZIC-HILIC column containing sulfobetaine as a ligand and a ZIC-cHILIC column containing phosphorylcholine as a ligand. The optimizing procedure was performed to achieve sufficient separation of the analytes in an acceptable time. Therefore, the effect of the amount of organic modifier in mobile phases and salt concentrations and the pH of the aqueous phase on the separation of pterin derivatives was studied. Under the optimized separation conditions (column ZIC-HILIC, mobile phase composition 83/17 (*v*/*v*) acetonitrile/10 mM ammonium acetate, pH 6.8) the method was used for the analysis of extracts from the integument of *Pyrrhocoris apterus.* The extracts were obtained using five different extraction systems, namely acetonitrile, methanol, dimethyl sulfoxide (DMSO) and two types of so-called deep eutectic solvents containing a mixture of choline chloride and lactic acid (DES 1) and a mixture of choline chloride and ethylene glycol (DES 2). DMSO was shown to be the most effective extractant, with a total of eight extracted derivatives from the twelve studied, and in the opposite, the least suitable extraction system was one of the so-called deep eutectic solvents, namely DES 1, with four extracted derivatives. The conditions for tandem-mass spectrometric detection of drosopterin were found and its pilot separation was performed.

Key words

pterins, hydrophilic interaction liquid chromatography, tandem-mass spectrometry, ZIC-HILIC and ZIC-cHILIC columns