ABSTRACT

Nowadays High Pressure Liquid Chromatography is the most important and the most frequently used separation technique that enables qualitative and quantitative analysis of the complex mixtures. One of its variants is Hydrophilic Interaction Liquid Chromatography. Lately this technique has experienced an increasing boom particularly, due to its ability to improve separation of very polar and hydrophilic compounds.

The adeninribonucleotides (ATP, ADP, AMP, cAMP), creatine and phosphoceratine are among these highly polar compounds that are of great importance not only for human body. Number of analytical methods has been developed and published for the analysis of these compounds; however HPLC methods using the reverse phases have been dominated. The basic overview of these methods is a part of this diploma thesis.

The experimental part of this thesis is focused on the development of chromatographic conditions suitable for the simultaneous analysis of ATP, ADP, AMP, cAMP, creatine and phosphocreatine in HILIC mode. The best separation was achieved on ZIC®-HILIC (150 x 2,1 mm; 3,5 μm; 200 Å) column using a binary mobile phase and gradient elution. The mobile phase consisted of the A component, (2 mM ammonium formate, pH 6,45) and the B component (90% of acetonitrile, 2 mM ammonioum formate) that were mixed together. The UV detection was performed at the wavelengths of 254 nm for ATP, ADP, AMP, cAMP and at 220 nm for C and PC.