Abstract

The aim of the diploma thesis was to find optimal conditions of high pressure liquid chromatography for the detection and quantification of two common nucleotides, namely adenosine diphosphate and adenosine triphosphate, as well as to perform an analysis of these in real life samples of citrus fruits and plant extracts. Further aim of the project was to determine the limits of detection and quantification of adenosine diphosphate and adenosine triphosphate under the optimized conditions and using these to compare the sensitivity of given detectors.

To achieve this HPLC-UV, capillary HPLC-DAD and HPLC-MS apparatus were used. With the help of HPLC with UV detection and capillary HPLC with diode array detector, the calibration curves of the mixture of analytes were measured and the limits of detection as well as quantification of adenosine diphosphate and adenosine triphosphate were determined. Separation of the analytes up to the base line using HPLC-UV and capillary HPLC-DAD was achieved under the conditions of ion pairing chromatography. Column C_{18} was chosen as an appropriate column. The mobile phase included phosphate buffer, acetonitrile and tetrabutylammonium bisulphate as an ion pairing reagent. The separation was performed with gradient elution. Conditions for analysis using LC-MS were determined. Using high pressure liquid chromatography with mass spectrometry, separation of analytes was achieved using column C_{18}, phenyl-hexyl column and Zic HILIC column. The mobile phase flowing through colony C_{18} included 99:1 (v/v) acetonitrile/ammonium formate with pH of 6.7. An appropriate mobile phase for the phenyl-hexyl column was acetonitrile 95:5 (v/v) acetonitrile/ammonium formate (pH 6.7). Flowing through the Zic HILIC column was a 70:30 (v/v) mixture of acetonitrile/ammonium acetate with pH of 5.35.

We were able to determine conditions of the HPLC for ADP and ATP analysis in real life samples. The sensitivity amongst given detectors was compared and correlated to those published in literature. The LOD and LOQ of analytes found are comparable, in some cases slightly higher than stated in literature on similar methods of detection of ADP and ATP. The sensitivity of mass spectrometer seems to be higher than that of the UV detector and the diode array detector. The last two reach similar sensitivity values.