# Abstract

## Optimalization of HPLC conditions for determination of biological active compounds

Diploma thesis

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Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosin monophosphate (AMP), creatine (C) and phosphocreatine (CP) are polar substances of great importance not only for the human body. In this work HPLC / UV method which allows the separation of these substances in isocratic elution was developed. As a suitable stationary phase zirconia column was chosen. The main advantage in comparison with the traditional silica columns is stability in the whole range of pH as well as at temperatures up to 200 ºC.

The best separation was achieved on a ZirChrom® - PHASE column by using a binary mobile phase with flow rate 1 ml/min - Part A: 10 mM hydrogenphosphate buffer, pH 7 and Part B: 100% acetonitrile. The detection was performed by using a UV detector at wavelengths of 254 nm for ATP, ADP, AMP, cAMP and 215 nm for C and CP. The final retention time valves under these conditions were: cAMP = 2.1 min, C = 3.7 min, AMP = 4.7 min, CP = 6.4 min, 10.7 min = ADP, ATP = 27.9 min. In the analysis of cardiac tissue the sample was mechanically homogenised with 57 µl of 100mM EDTA first and then 0.5 ml icecold 0.66M $HClO_4$ was added. 1 ml 0.66 M $K_2HPO_4$ was added after the proces of deproteination and then the sample was centrifuged for 5 min at 10,000 rpm. Afterwards a supernatant was removed and 20 µl of this supernatant were injected onto the column. Adequate stability of the sample was provided by the maintenance of temperature 4$^oC$ throughout the whole proces of the sample preparation.