**Abstract**

A coupling of Ultra High Performance liquid chromatography with mass spectrometry provides a technique, which is rapid and sensitive. This thesis is focused on the use of UHPLC-MS for the determination of ascorbic acid (AA) and dehydroascorbic acid (DHA).

AA is a small polar molecule that acts as an antioxidant. After oxidation AA creates DHA. AA/DHA ratio is an indicator of a redox state of organism. For the determination of AA and DHA several methods have been developed, which usually do not allow the simultaneous analysis, but require multistep subtraction procedure.

The optimization of UHPLC-MS method for the determination of AA and DHA include the choice of mobile and stationary phase and mass spectrometry detector set-up.

The choice of appropriate conditions depended mainly on retention time and the detector response. The effect of stationary phase, concentration, pH, composition of mobile phase on retention of AA and DHA was observed. Effect of mobile phase on stability of AA and DHA was observed as well.

BEH Shield RP C18, BEH HILIC and BEH Amide column were compared. The best results were achieved on column BEH Shield RP C18. Measurements with 0.1%, 0.05%, 0.01% formic acid, 0.1%, 0.05%, 0.01% acetic acid, ammonium formate at pH 3.5 and amonium acetate at pH 4.4 and 6.8 as a water component were carried out. The best results were achieved with ultrapure water. Organic part of mobile phase was 65:35 acetonitrile (ACN).

Repeatability test was performed at optimized conditions using SIM experiment. Relative standard deviation of peak area for AA was 1.63% and for DHA was 8.95%. Calibration curve was measured in the range 1-500 μg/ml. Correlation coefficient of calibration curve was for AA 0.9955 and for DHA was 0.9985.