Abstract

This thesis was focused on the determination of ascorbic acid (AA) using Hydrophilic Interaction Chromatography (HILIC) approach and the comparison of HILIC stationary phases.

Determination was carried out using three types of detection. To optimize the method ultraviolet (UV) detection was used. For the determination of AA and DHA (dehydroascorbic acid) on different stationary phases aerosol based detectors Charged Aerosol Detector (CAD) and Nano Quantity Analyte Detector (NQAD) were used.

During method optimization suitable conditions for the determination of AA and DHA have been defined. Aqueous part of the mobile phase was formed by water, acetic acid or ammonium acetate buffer of increasing strength and different pH. Organic part was formed by acetonitrile. During the optimization
the influence of gradual changes of water and organic part has been monitored.

Compared stationary phases were Atlantis T3 and those specially developed for the determination by HILIC method - Luna HILIC and ZIC-HILIC. In the case of Luna HILIC and Atlantis T3 both analytes were not separated from the dead retention time. On ZIC-HILIC column separation of substances occurred, but peaks were too broad. None of these columns has been evaluated as suitable for the simultaneous determination of AA and DHA.

A part of this thesis was to verify the possibility of using the Corona CAD detector. CAD enables simultaneous detection of AA and DHA in terms of finding a suitable chromatographic system.

New type of aerosol based detector NQAD was tested as a new option for simultaneous determination of AA and DHA. Unfortunately low reproducibility has been observed. It was found that the detector was not robust
enough and it was very sensitive to the use of buffers as the aqueous component of mobile phase, type of the column and it required the use of chemicals of the highest purity.

A HILIC method using Obelisc R was applied for the method validation under the conditions which can be applied in connection with NQAD in the future. Recovery, precission of the method, linearity and robustness were measured.