Abstract

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Title of the diploma thesis: Development of UHPLC-DAD method for separation of phenolic substances in plant material and extraction optimization

This diploma thesis deals with development of UHPLC-DAD method for separation of selected phenolic substances (arbutin**,** chlorogenic, neochlorogenic, cryptochlorogenic, isochlorogenic A and caffeic acid, cynarin, catechin, epicatechin, rutin**,** hyperoside, hirsutrin, reynoutrin, guaiaverin, quercitrin and quercetin).

UHPLC analysis of these phenolic substances took place on a Luna® Omega Polar C18 100 (150 x 2.1 mm; 1.6 µm) column with fully porous particles. As a part of the optimization method, a column with core-shell particles (Kinetex® Polar C18) and a column with a hybrid stationary phase (YMC-Triart C18 ExRS) were also tested. The sample injection volume was 2 µl, the flow rate has been set to 0.3 ml/min and temperature of the column has been set to 30 °C. As a mobile phase ultrapure water acidified with acetic acid to pH 2.8 in combination with acetonitrile has been used. The total analysis time was 17.5 min and detection was performed using a DAD detector at wavelengths 254, 280, 320 and 365 nm.

The optimized UHPLC-DAD method was used to determine the content of selected phenolic substances in pear leaves. Extractions were performed in an ultrasonic bath using ethanol and methanol at various concentrations as extraction solvents. The extraction time was also optimized. The largest amount of phenolic substances was in the extraction solvent with 20% ethanol in water after 30 min of extraction.