

Abstract

Charles University

Faculty of Pharmacy in Hradci Králové

Department of Analytical Chemistry

Candidate: Bc. Stanislava Košková

Supervisor: Doc. PharmDr. Hana Sklenářová, Ph.D.

Consultant: Ing. Radek Vávra, Ph.D. (VŠÚO Holovousy)

Title of diploma thesis:

Evaluation of selected substances in fruit by HPLC-DAD-CAD method

This diploma thesis was focused on determination of seven phenolic compounds, namely gallic acid, chlorogenic acid, epicatechin, rutin, phloridzin, quercetin, phloretin in twenty apple cultivars, that have been delivered by the Research and Breeding Institute of Pomology Holovousy s.r.o.

For this purpose HPLC separation in reverse mode was used, using the column Omega Polar C 18 (150 mm x 4.6 mm; 5 μ m). As the mobile phase the mixture of an ultrapure water acidified with the acetic acid to pH 2.8 and acetonitrile was used. A linear gradient was chosen with a gradual increase of the organic component from the initial 10 % to 50 % during 10 min of separation. This was followed with column equilibration – up to 12.50 min to 10 % organic component. The overall separation time was 12.50 min at a flow rate of 1 ml/min. Column temperature was set to 30 °C. Detection was performed using DAD (254, 280, 320 nm) and CAD detector (nebulizer temperature 25 °C).

Part of this thesis is system suitability test that includes: resolution (R_s), symmetry factor (A_s), repeatability of the retention times and peak areas and peak capacity. In addition, the following validation parameters were evaluated: linearity, detection limit and quantitation limit, selectivity, precision and accuracy.

Validated HPLC-DAD-CAD method was applied for comparison of the phenolic profiles of the tested apple cultivars. Processed plant material includes selection of 3 fruits of each cultivars, removing the cores, followed by homogenization of the pulp with the peel, extraction with methanol acidified with acetic acid, ultrasound-assisted extraction, followed by centrifugation and filtration.

In real samples, 4 of the 7 phenolic substances were found. These were chlorogenic acid, epicatechin, rutin and phloridzin. Most of these substances were found in cultivars HL 2010, HL 207, Meteor, HL 1199 and HL 2350. No analytes were detected in cultivars HL 1343, HL 72 and Topaz.