

## **ABSTRACT**

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Jana Majerčíková

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

Title of the diploma thesis: HPLC separation of phenolic substances in plant material

This diploma thesis is focused on development and optimization HPLC-DAD method for determination of 17 phenolic compounds (gallic acid, protocatechuic acid, 4-aminobenzoic acid, chlorogenic acid, catechin, caffeic acid, epicatechin, vanillin, p-coumaric acid, ferulic acid, salicylic acid, rutin, o-coumaric acid, quercitrin, myricetin, quercetin and kaempferol) in apricot variety Betyнка.

In the process of optimization several types of stationary phase and gradients of mobile phase were tested. For the analysis of real samples guard column Security Guard™ Polar C18 (4 x 2.0 mm, 3 μm) and column Luna Omega Polar (150 mm x 2.1 x 3 μm) were selected. As mobile phase the mixture of ultrapure water acidified with the acetic acid to pH 2.8 and acetonitrile was used. For the analysis the gradient elution with initial isocratic step was used, total analysis time was 17 minutes. The flow rate was 0.6 ml/min and the injection volume 1 μl. The detection was performed by DAD spectrophotometric detector at wavelengths of 254, 260, 280 and 320 nm.

Validate HPLC-DAD method was applied for comparison of phenolic compound content in the original material and after 10, 20 and 30 days storage in 1-methylcyclopropene. Further, apricots treated with pesticides with control group without treatment pesticides were compared. For the extraction methanol with 0.1% acetic acid was used.

In real samples 4 phenolic compounds – chlorogenic acid, catechin, epicatechin and rutin were detected and quantified. The highest levels of concentrations were found out in original samples and during the storage the changes of these original levels were observed.