ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Zuzana Jandorová

Supervisor: Prof. PharmDr. Lucie Nováková, Ph.D.

Title of the diploma thesis: A study of separation conditions and stability of selected phenolic compounds

The goal of this diploma thesis was to determine the most suitable separation conditions for the analysis of ten phenolic compounds by the ultra-high performance liquid chromatography with PDA detection. The thesis is focused mainly on the separation of critical pair tamarixetin and isorhamnetin. Another goal was to determine the short-term stability of all tested compounds for 24 hours.

Analyses were performed using the ACQUITY UPLC chromatographic system with PDA detector at a wavelength of 275 nm. The first step in the study of separation conditions was the screening of 17 columns from several different manufacturers. Tested columns included ACE Excel C18, BEH C18, CSH C18, Arion Plus C18, Ascentis Express C18, Atlantis Premier BEH C18 AX, BEH Shield RP18, Cortecs Shield RP18, Ascentis Express RP-Amide, BEH Phenyl, CSH Phenyl-Hexyl, Kinetex Biphenyl, ACE Excel C18-AR, ACE Excel C18-PFP, CSH Fluoro-Phenyl, Ascentis Express F5, and Kinetex PFP. During the screening, 3 organic modifiers (acetonitrile, methanol and acetonitrile with methanol 1:1) were tested in combination with 2 aqueous components (0.1% formic acid and 0,1% acetic acid). The second step was the optimization of separation conditions on the 3 most suitable columns, namely Ascentis Express RP-Amide, Kinetex Biphenyl, and ACE Excel C18-PFP. The effect of gradient curves, the gradient elution setting, the separation temperature and the analysis time were optimized.

The best results of separation of the analytes was achieved on the Kinetex Biphenyl column under the conditions: methanol with 0.1% formic acid, gradient program 15-98 % MeOH within 7 minutes followed by 2 minutes of equilibration, separation temperature 40 °C. These conditions were also used to measure the short-term stability of individual phenolic compounds.

The stability was measured every hour for 24 hours. The change in peak area over time and the precipitation of compounds during storage in the autosampler were monitored. The measurements were performed at 10 °C, 15 °C, and 20 °C. The compounds were dissolved in water with HCOOH and 20% methanol with HCOOH, which was chosen based on a comparasion of the effect of the solvent on the peak shape. Each compound was subjected to 6 measurements. PAA, 4MC, HFP and HVA proved to be stable compounds under all tested conditions. For these compounds, the concentration didn't decrease below the limit of 95 %. PG, HFA and RUT were stable only under certain conditions. QCE, TAM and ISO showed problematic behaviour, with precipitation in the solution.