Abstract

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Title of Diploma thesis: *Evaluation of properties of new „fused – core" analytical columns in UHPLC system and a comparison with the BEH UPLC analytical columns*

This thesis was focused on testing properties of new analytical columns filled with porous shell particles (so-called fused-core) in UHPLC system and a comparison with the BEH UPLC analytical column.

The following analytical columns were tested: Kinetex PFP particle size of 1,7 µm and 2,6 µm (Phenomenex) and Ascentis Express Phenyl-Hexyl particle size of 2,7 µm (Sigma-Aldrich), which are columns with porous shell particles and Acquity BEH Phenyl column with totally porous particles size of 1,7 µm.

To compare the properties of the columns, 7 estrogen steroids were used using PDA detector at a wavelength of 280 nm for detection. Measurements were carried out using isocratic elution. The Mobile phase consisted of ultrapure water and acetonitrile.

Columns were evaluated in terms of efficiency, peak symmetry, resolution and the back pressure, which was generated on the columns. The efficiency of the columns was expressed as a number of theoretical plates and by van Deemter plots. Further, the influence of increase in temperature and flow-rate on the separation of estrogen steroids was monitored. With increasing temperature and flow-rate there was a significant reduction in analysis time. Columns were also compared in terms of speed of analysis.

The best performance expressed as a number of theoretical plates was achieved with the Acquity BEH Phenyl column and a slightly lower efficiency was evaluated on the Ascentis Express Phenyl-hexyl column. While there was no significant difference in efficiency of these two columns, comparing the values of back-pressure, much higher increase was achieved with the Acquity BEH Phenyl column compared to the Ascentis Express column. The fastest separation was obtained on Kinetex PFP column particle size of 1,7 µm. Together with the Kinetex PFP particle size of 2,6 µm these columns were evaluated to have worse efficiency and significant by increased in back-pressure. The values of back-pressure generated on the Kinetex PFP column particle size of 1,7 µm filled with porous shell particles were even higher than on Acquity BEH Phenyl column, which is filled with totally porous particles.