ABSTRACT

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Title of Diploma Thesis: Evaluation of the influence of various solvents on column storage for supercritical fluid chromatography

The diploma thesis deals with the evaluation of the influence of various solvents on column storage for supercritical fluid chromatography. It is focused especially on column aging which is manifested as reduced retention time and the change in selectivity of a stationary phase when stored for a certain period of time.

The experiments were performed using the ACQUITY UPC^2 SFC system with the PDA detector. The detection took place at wavelength of 225 nm for the mixture of neutral compounds and at wavelength of 230 nm for the mixture of ionisable compounds. The ACQUITY UPC^2 BEH 1.7 μm and the ACQUITY UPC^2 BEH 2-EP 1.7 μm columns with dimensions of 3.0 x 100 mm (Waters, USA) were used as stationary phases. After the separation, these columns were always flushed with different solvents and stored, namely in CO_2, isopropanol, acetonitrile and hexane in which they were stored until the next use. CO_2 with methanol as an organic modifier was used as a mobile phase for the mixture of neutral compounds. CO_2 and methanol with the addition of 20 mM ammonium formate was used for the mixture of ionisable compounds. The flow rate of mobile phase was 2.5 ml/min and the separation of analytes was performed by gradient elution. The column temperature was maintained at 40°C and the ABPR pressure was set to 2000 psi.

Column aging was manifested on all columns, when they were used and stored long-term, but this effect was manifested in a different extent and speed. Among the solvents used for the column storage, acetonitrile showed the best results on both stationary phases, because when columns were stored in this solvent, the column aging was manifested more slowly than with other solvents.

Key words: supercritical fluid chromatography; column aging; BEH; BEH 2-EP columns; CO_2; acetonitrile; isopropanol; hexane