

ABSTRACT (EN)

The dissertation thesis is focused on major trends in high performance liquid chromatography such as miniaturization of separation systems in hyphenation with high-sensitivity detection or characterization of new types of stationary phases for the separation of polar compounds in systems suitable for mass detection. Application of recently developed stationary phases in hydrophilic interaction liquid chromatography (HILIC) is also considered.

Capillary liquid chromatography with tandem mass spectrometry (cLC-MS/MS) method was developed for determination of five estrogenic pollutants in samples of water. Several new sorption materials for solid phase extraction (SPE) were compared to obtain sufficient recovery of all the tested analytes. Discovery DSC-18Lt column provided the highest recovery (95 – 100 %). The optimized cLC-MS/MS with SPE was used for determination of estrogens in water samples in the order of units to tens of ng/L.

HILIC separation systems with silica gel, cyclofructan and isopropyl cyclofructan modified silica stationary phases were tested and compared. Ability to donate protons and dispersion interactions are the main interactions that affect retention in HILIC with cyclofructan-based columns while they are less important in separation systems with bare silica stationary phase. Improved separation performance and selectivity of cyclofructan-based stationary phases, as compared with unmodified silica gel, for separation of peptides was demonstrated in HILIC.

Two amide-based HPLC columns XBridge™ Amide column and TSK gel Amide-80 column were characterized in detail and compared in HILIC mode. HPLC separation systems with amide-based columns were characterized by simple chromatographic tests, linear free energy relationship model (LFER) and newly designed approaches. The amide-based columns showed certain differences in retention, selectivity and efficiency.

A new separation method involving HILIC with tandem mass spectrometric detection was developed for the analysis of polar pterines in the integuments of heteropteran insect species. The optimized conditions for the separation of pterines consisted of ZIC-HILIC column and mobile phase composed of acetonitrile/5 mM ammonium acetate, pH 6.80, 85/15 (v/v), flow rate 0.6 mL/min and column temperature 30 °C. The method was applied to the analysis of pterines in the integuments of *Graphosoma lineatum* and *Graphosoma semipunctatum*.