

ABSTRACT (EN)

This work is focused on development of new analytical methods involving modern solid phase extraction (SPE) for sample purification and pre-concentration, state-of-art ultra high-performance liquid chromatography (UHPLC) for separation of analytes and detection using diode-array detection (DAD) and/or time-of-flight mass spectrometry (ToFMS). The major part of the results has been published in prestigious international journals. The relevant yet unpublished results are included in the thesis as well. Besides a model comparison of various column chemistries used for “ultra-high performance” separations, some meaningful applications were developed. While one part of applications deal with determination of antibiotics as environmental contaminants that are responsible for the development and spread of microbial resistance, the second part is concerned with analyzing and finding of potential natural source of novel antimicrobial agents that can be further used as new antibiotics.

The first part of the thesis was focused on the determination of residual antibiotics in different matrices (liquid hog manure, wastewater, surface water). The mostly prescribed antibiotics in both human and veterinary medicine were chosen as analytes in this study employing analytical methods consisting of UHPLC-DAD and UHPLC-ToFMS together with SPE or liquid-liquid extraction (LLE). The methods were validated and the proper calibration techniques were employed for quantification. The developed procedures were applied for analysis of real environmental samples from different localities in the Czech Republic and the results revealed the occurrence of antibiotics in the majority of the tested samples. The liquid hog manure was positive for tested tetracyclines at concentrations up to 6 mg kg^{-1} . All tested water samples were positive for studied antibiotics at concentrations ranging from 5 ng L^{-1} to 1290 ng L^{-1} .

The second part of the thesis was focused on development of analytical methods useful for study of secondary metabolites produced by fungi of genus *Geosmithia*. The probability of the employment of the secondary metabolites' production for the characterization of these fungi was expected. Moreover, these symbionts represent the potential reservoir of bioactive secondary metabolites with various antimicrobial activities. A new UHPLC-DAD-ToFMS method was developed for chemical fingerprinting of extracellular secondary metabolites in fermentation broth of these fungi and was applied for analysis of 48 *Geosmithia* strains. Various SPE chemistry and LLE procedures were tested resulting in employment of strong cation-exchange mixed-mode polymeric sorbent (Oasis MCX) for extraction of secondary metabolites. Strong correlation between the UHPLC-DAD-ToFMS fingerprints and the taxonomical identity of *Geosmithia* spp. was revealed. These results qualify the method as a chromatographic fingerprinting tool for characterization of fungal strains based of the analysis of their secondary metabolites.

The subsequent chromatographic screening for bioactive secondary metabolites employed both UHPLC-DAD-ToFMS and HPLC-UV methods. Among the tested strains, the secondary metabolites characterized by wide antimicrobial spectrum were revealed and these compounds were determined using the developed analytical methods. This fact confirmed that the introduced LC methods represent a useful tool for the chromatographic screening for bioactive secondary metabolites and that the *Geosmithia* fungi are promising reservoir of interesting antimicrobial compounds.

The third part of the thesis was aimed on the approaches how to improve the efficiency and speed of analysis of microbial secondary metabolites. The recently introduced columns packed with superficially porous particles (represented by Kinetex C18 column) were tested and compared with fully porous particles (represented by Acquity BEH C18 sub- $2\mu\text{m}$ particle column) in UHPLC system under both acidic and alkaline conditions using gradient elution program. The Kinetex C18 column was found to be a suitable alternative to Acquity BEH C18 column. The great advantage of the Kinetex C18 column is the lower column backpressure that enables its use on conventional LC system. However, the limitation of employment of Kinetex C18 column under alkaline conditions was observed with respect to its decreasing performance with the growing number of injections on the column.