Abstract

This dissertation thesis composes of two parts; the first part focus on the characterization of trypsin, enzyme frequently used in proteomic research for the investigation and identification of protein sequences, and its peptide digestion kinetics. The second part is aimed to the enantioseparations of biologically active compounds.

First part of this project focus on tryptic digestion of synthetic peptides and the development of HPLC method for the identification of synthetic peptides and their fragments. Using the in-solution digestion and HPLC method, relative kinetic constants were determined for problematic sequences. Amino acids responsible for the decrease in trypsin catalytic activity and their location towards the cleavage site were studied. Certain slight exopeptidase activity of trypsin was noted, especially at the end of peptide chain. Furthermore, three columns with immobilized trypsin used in HPLC were compared concerning their catalytic activity. The immobilization of enzymes on solid support is used to elevate the amount of enzyme present during digestion and to assure better repeatability and reproducibility of obtained results. Activity of a new trypsin column synthesized at the University of North Carolina at Chapel Hill was compared to two commercially available trypsin columns. *N*α-benzoyl-L-arginine 4-nitroanilide hydrochloride was used as a substrate and the separation conditions tested included the use of various buffer pH, flow rates and temperatures. The results showed that the newly synthetized trypsin column showed much higher activity than the commercial ones, especially at pH 9.0 measurements.

The second part deals with HPLC and CE chiral separation methods for the determination of tryptophan and its unnatural derivatives. These analytes can be used for instance in pharmaceutical industry for various purposes. In the case of CE measurements, native cyclodextrin and its derivatives were used as chiral selectors. The optimization of HPLC method required application of various chiral stationery phases. Stationary phases based on derivatized cyclodextrins, cyclofructans, teicoplanin and polysaccharides were tested. All the analytes were baseline separated. Based on the obtained results separation mechanisms were discussed. This part also includes a vast research on the enantioseparation of pharmaceuticals, amino acids and other chiral compounds by HPLC, SFC, GC and EKC separation methods.