Non-Denaturating Separation and Detection of Proteases by Means of LC-Post-Column Reaction UV/vis

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Enzymatic conversions play a crucial role in the regulation of all processes of life. As biocatalysts in industrial processes or targets in drug discovery, enzymes originating from natural sources are getting more and more important. In particular, proteases like thrombin, coagulation factors or angiotensin converting enzyme (ACE) play a major role in the regulation of blood coagulation and blood pressure and are therefore important targets for the development of drugs and diagnostic applications.

Protein analysis is commonly carried out by gel electrophoretic techniques like SDS-PAGE or IEF. Chromatography is mainly used to fractionate complex biological samples by size in fast protein liquid chromatography (FPLC). Size exclusion chromatography (SEC) and ion-exchange chromatography (IEC) allow the separation under non-denaturating conditions and are therefore applicable for separation of native enzymes. In all common screening techniques for enzymatic activities, the separation and determination of enzymatic activity are performed in two separate steps. Thus, the screening is time consuming and not suitable for large numbers of natural samples. Online methods, known for inhibitoric assays [1], could be a promising alternative.

Therefore, a liquid chromatographic method with on-line acitivity determination of proteases was developed. The separation was carried out by IEC and SEC respectively, without losing the proteolytic activity. The eluting proteins first pass a UV detector (280 nm), and then are subjected to a reaction with *p*-nitro aniline (pNA) labelled peptides as substrates. In a knitted reaction coil, the protease catalyses the reaction under release of the coloured pNA. Measurement of the enzymatic activity is performed using a second UV detector at 405 nm, detecting the pNA at the end of the reaction coil. The developed method was successfully applied to the separation of model proteases from animals (trypsin), plants (ficin) and bacteria (pronase).

[1] de Jong, C. F., Derks, R. J., Bruyneel, B., Niessen, W., and Irth, H. (2006). High-performance liquid chromatography-mass spectrometry-based acetylcholinesterase assay for the screening of inhibitors in natural extracts. J Chromatogr A *1112*, 303-310.