

Abstract

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Title of Diploma Thesis: Analysis of biopharmaceuticals by liquid chromatography.

Optimalization of sample preparation.

This diploma theses is focused on development and optimalization of prepared samples in acidic pH for peptide mapping at the same time with minimization creation of undesirable peptide modifications during preparation.

Within the method of optimalization there were prepared several samples which were different in preparation process, use of chemicals or enzymes and also different condition of incubation. Enzymes used for preparation of samples were trypsin, endoproteinase Lys-C, endoproteinase Glu-C, endoproteinase Asp-N and endoproteinase Sap-9. At diploma theses were also used commercially available sets for preparing samples in acidic pH and then results were compared. Samples were analyzed by UHPLC-UV and LC-MS methods. Results from LC-MS analysis were processed by proteomics softwares like FragPipe and Byonic (Protein Metrics), which could be evaluated processes for digestion and preparation of samples in acidic pH, described their advantages or disadvantages

For the complete comparison and evaluation of the best processes for prepared samples for peptide mapping was performed preparation samples both manual and online at a column with immobilized trypsin STYROSZYME TPCK-TRYPSIN (2,1x150 mm).