Abstract

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Title of graduation thesis: Peptide Mapping of Biopharmaceuticals Using CE

This work focuses on optimization of capillary electrophoresis (CE) method for the peptide mapping. and enzymatic digestion of proteins using trypsin. Myoglobin from equine heart and a reference antibody were used as substrates. Two digestion approaches were compared: a conventional tryptic digestion in solution and digestion with a commercial SMART Digest Trypsin Kit that is based on immobilized trypsin. The digestion was monitored with RP-HPLC. After a short optimization of tryptic digestion, a number of experiments were carried out to determine the optimal conditions for peptide mapping by means of CE. All the measurements were carried out using a PVA-coated capillary of 50 µm internal diameter, effective length of 56 cm and total length of 64.5 cm. Samples were applied hydrodynamically at a pressure of 50 mbar, the separation voltage was 30 kV, the temperature of the capillary was set to 20°C. The analytes were detected with UV-VIS detector at 200 nm. The effect of BGE type and concentration (30, 60, 90, 120, 150 mM formic acid a 0.5%, 1%, 1.5%, 2% acetic acid), injection time (20, 30, 40, 50, 60, 70, 80s) and the sample concentration was examined. Dynamic pH junction was used for on-line preconcentration of the peptides.

The best peptide maps of the reference antibody were achieved with 60s injection of 0.1075 µg/µl solution of antibody digest dissolved in 10 mM solution of ammonium bicarbonate, pH 8.5. Formate buffer (90 mM, pH 3) was used as the BGE. The most effective digestion of the reference antibody was achieved using the commercial SMART Digest Trypsin Kit for 180 min.