ABSTRACT

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Title of diploma thesis: Pros and cons of using elevated temperature for peptide separation using reversed phases

Nowadays, powerful mass spectrometers are available for bottom-up LC-MS proteomic analyses. They allow to identify up to several thousand proteins in one bottom-up analysis. It has been recently demonstrated, however, that their capabilities cannot be fully exploited without significant improvements in peptide separation. One of the easiest, but often neglected, ways to enhance peptide separation efficiency is to increase column temperature. Elevated column temperature may contribute to more efficient separation especially by increasing low diffusivity of peptides. On the other hand, stability of both a stationary phase and peptides limits increasing column temperature to values affording maximum effect on separation efficiency. In this thesis, we explored the effect of increased column temperature on peptide separation efficiency using columns packed either with superficially porous particles or with fully porous particles. Elevated column temperature narrowed peaks for peptides eluted from fully porous particles whereas peak widths of peptides eluted from superficially porous particles did not changed remarkably. Subsequently, using a protein model we examined on-column thermal stability of peptide bonds. Surprisingly, combination of 0.1% formic acid in mobile phases and temperature 45 °C led to detection of first degradation product already. Lastly, we explored on-column chemical stability of amino acid residues at elevated temperatures. Some modifications were expectable, but we also identified some atypical modifications. In conclusion, our results demonstrate that depending on type of particles, temperature can improve peptide separation efficiency. However, column temperature must be used wisely, particularly during long LC-MS analyses.

Keywords: Proteomic analysis, peptides, temperature, stability, separation efficiency, reversed phase, LC-MS