ABSTRACT

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Title of diploma thesis: **A high-temperature LC-MS method for bottom-up proteomic analyses with reduced artifacts**

Proteomic bottom-up LC-MS analyses need more efficient chromatographic separation to keep up with the advances in mass spectrometry and fully exploit the potential of state-of-the-art MS instruments. Elevation of column temperature represents one of the most powerful and cost-effective means for improvement of separation performance. However, high temperature also promotes in-column modification of peptides, putting a spoke in the wheel of sophisticated proteomic analyses.

The current method aims to minimize the formation of temperature-related artifacts via a novel high-flow trap-elute setup with differential column heating. The trap-elute setup reduces the time peptides spend in the heated separation column, resulting in fewer generated artifacts. This mitigates an important drawback of the high column temperature. At the same time, it does not diminish its positive effect on the separation performance. Consequently, the utilization of the elevated column temperature becomes more profitable.

The proposed method reduced the artifact abundance among identified peptides in an exploratory single-shot analysis of a human cell line proteome. It also maintained the number of identified unique peptide sequences comparable to the direct injection configuration. The trap-elute setup was also successfully integrated into a mimicked multi-attribute method for the characterization of therapeutic proteins, where it again reduced analysis-related modification in structure regions with a critical influence on product quality.

Keywords: bottom-up proteomics, liquid chromatography, mass spectrometry, high temperature, artifacts, biopharmaceuticals.