ABSTRAKT

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Title of diploma thesis: Online chemical cleavage of proteins for rapid analysis of biopharmaceuticals and identification of bacteria

In 2023, Dr. Lenčo and his team published a fast and easy method of liquid chromatography coupled with mass spectrometry for protein analysis. The method consisted of the cleavage of proteins in a subcritical acidified mobile phase. This method demonstrated success in the rapid detection of ricin. Other potential applications of the method may include the rapid characterization of protein biopharmaceuticals and the rapid identification of bacteria.

Protein biotherapeutics based on monoclonal antibodies are highly effective and safe. These drugs are particularly used to treat serious diseases such as oncological and autoimmune disorders. The increased number of approved antibody-based drugs compared to conventional chemical therapies in recent years demonstrates their significant therapeutic potential. A certain disadvantage is their complex structure and the related need for thorough analysis and quality monitoring, where rapid online sample preparation could bring certain advantages.

Currently, the main method in clinical diagnostics of bacteria is MALDI-TOF mass spectrometry, which can record specific spectra even for related bacteria. However, these spectra do not provide any sequence information. If the mass spectra contained sequence information, the identification of bacteria would become much more certain. This would also remove the dependence on libraries of MALDI-TOF spectra.

This thesis focuses on further optimization of the method of protein cleavage in the subcritical acidified mobile phase and the extension of its possible applications. To this end, we have used this method to analyze five monoclonal antibody-based

biotherapeutics (panitumumab, bevacizumab, cetuximab, trastuzumab, and aflibercept) and three bacterial strains (*B. subtilis*, *E. coli*, and *S. aureus*).

During optimizing the method, the original dry bath was replaced by a heated block. Although this transition resulted in a lower number of identified peptides, it greatly improved the convenience and safety of the work. The method demonstrated some applicability in the analysis of antibody-based biologics, but its performance was severely limited by the occurrence of undesirable peptide modifications. In the field of bacterial identification, the greatest challenge was to find a universal procedure for efficient and rapid preparation of whole cell lysate. The best results were obtained using a lysis solution consisting of 100% formic acid.

Since the formation of unwanted modifications during online protein cleavage in the subcritical acidified mobile phase severely limited the possibility of effective quality control of protein biotherapeutics, the method was redirected to applications in rapid and sequence-specific identification of bacteria. Experimental results show that online protein cleavage in subcritical acidified mobile phase may offer an interesting alternative to traditional MALDI-TOF mass spectrometry-based diagnostics.

Keywords: proteins, peptides, online cleavage, antibody biomedicines, bacterial identification, LC/MS