ABSTRACT

Charles University
Faculty of Pharmacy in Hradec Králové
Department of Analytical Chemistry

Candidate: Mgr. Lenka Popovská
Supervisor: Prof. RNDr. Petr Solich, CSc.
Consultant Supervisor: Mgr. Barbora Šalovská, Ph.D.
Title of the rigorous thesis: **Optimization of procedure for isolation of phosphorylated peptides from a peptide mixture by metal oxide affinity chromatography for mass spectrometry analysis.**

Protein phosphorylation is the key regulatory mechanism in cellular signalling. It is involved in most cellular processes that are regulated by a complex interplay of protein kinases and protein phosphatases and include proliferation, differentiation, and apoptosis. Defective or altered signalling pathways can lead to various diseases, and therefore it is very important to study protein phosphorylation in detail and try to understand it as much as possible.

Technological development of mass spectrometry (MS) in recent years has enabled a significant increase in the number of phosphorylation sites that may be identified in a sample. Increasing scan speed together with commercial availability of high resolution MS have improved quantity and quality of the acquired data, which can be obtained in a relatively short time. However, even high-end devices would not allow to comprehensively identify the phosphorylation sites in enzymatic digests of the samples if the analysis itself was not preceded by any of the techniques for the enrichment of phosphopeptides.

The aim of this thesis was to optimize the process for the isolation of phosphorylated peptides (from two differently complex mixtures obtained by tryptic digestion, which differ in molar ratios of standard phosphorylated and non-phosphorylated proteins) using Metal Oxide Affinity Chromatography (MOAC), which is based on high affinity of the phosphate groups to the metal oxides (especially TiO_2 and ZrO_2) in acidic medium, employing commercially available spherical particles Titansphere® of 5.0 and 10.0 μm.