

Development of Self-Packed Columns for (Phospho)Proteomics**Tóth, G.^{1*}, Tóth, E.², Ozohanics, O.², Borsos, K.¹, Turiák, L.¹, Drahos, L.^{1,2}**

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Phosphorylation is one of the most common post-translational modifications of proteins, and has important biochemical roles. It takes part in the fast on/off switch of enzymes and plays a role in the regulation of synaptic signal transmission. Due to the large variety and low concentration in comparison with the unphosphorylated peptides, the qualitative determination and quantification of phosphopeptides is a difficult analytical task therefore they cannot be quantified without additional phosphopeptide enrichment.

Self-packed columns allow the use of a wide range of the stationary phases, while being simple and economical. The aim of the presented research is to develop capillary column packing methods for proteomic and phosphoproteomic research, optimise the parameters for the packing and compare the resulted enrichment and purity with commercially available phosphopeptide enrichment kits.

We developed a protocol for the packing of reversed phase C₁₈ capillary columns. The reproducibility of the self-packed columns was examined with the separation of the tryptic digest of enolase and alpha acidic glycoprotein (AGP). The self-packed columns were characterized by good reproducibility (RSD < 1.1%), the columns are of good quality; the peaks are narrow (σ < 0.2 min) and the resolution is optimal with peak capacities near 100. Besides these, there is no shift in the retention times (< 0.075 minutes). We also made a comparison with a similar commercially available reversed phase capillary column. As an outstanding result, the separation of isomers of glycopeptides was possible using the self-packed columns, but not using the commercial column.

A detailed examination was performed for the maximum enrichment efficiency of TiO₂-MOAC and Fe-IMAC SPE spin columns using a set of model proteins and a phosphopeptide standard mix. We applied the optimized TiO₂ enrichment protocol to study the effects of sleep deprivation on the rat synaptosome.

Combining the two techniques, TiO₂ nanospheres were packed into a 250 μ m i.d. capillary creating a 10-cm long column. The packing method was optimized and the enrichment efficiency was tested. The preliminary results will be discussed in detail in the presentation.

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