

COMPARISON OF SOLID-PHASE EXTRACTION METHODS FOR PHOSHOPEPTIDE PURIFICATION

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Phosphorylation of proteins is a post-translational modification with a wide range of biological functions. Efficient purification of phosphopeptides is necessary for the nanoHPLC-MS/MS analysis of the phosphorylation in small amounts of biological samples. Our goal was to find the most efficient solid-phase extraction (SPE) method for small amounts of complex samples (0,5-1 µg).

For the method development, we used formalin-fixed paraffin-embedded (FFPE) lung cancer tissue samples. Following on-surface tryptic digestion of proteins, the resulting peptide mixture was purified using Thermo Pierce C₁₈ cartridges, and then the phosphopeptides were enriched using Thermo Pierce TiO₂ pipette tips. For the subsequent purification of phosphopeptides, four stationary phases (Thermo Pierce C₁₈ Columns, Thermo Pierce C₁₈ Spin Tip, Affinisep C₁₈ Spin Tip, Affinisep HLB Spin Tip) were tested with different solvent systems. Altogether, five SPE methods were compared using five parallel samples, unpurified samples were applied as a control. The nanoHPLC-MS/MS measurements were performed on a nanoAcquity HPLC (Waters) coupled to a Bruker Maxis II tandem mass spectrometry instrument (Bruker Daltonics). Peptide and protein identification were conducted using Byonic.

For each SPE purification method, the number of identified phosphopeptides, the GRAVY score distribution, the isoelectric point distribution of the identified peptides, the site distribution of the modifications, as well as, the number of modifications were compared. Most of the SPE methods performed similarly, however, the Thermo Pierce C₁₈ Spin Tip was the most efficient. With this SPE method, 6.7 % more phosphopeptides were identified than in the unpurified control samples. Comparing the overlaps of the identified phosphopeptides between the methods, approximately 50% of the phosphopeptides are identified only in one method.

For the analysis of protein phosphorylation of small amounts of complex tissue samples, the Thermo Pierce C₁₈ Spin Tip performed very efficiently.

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