INTRODUCTION

The requirement for robust and routine high-throughput sample preparation workflows has become a necessity as clinical proteomics research evolves. The workflows enable the parallel processing of large sample cohorts with the throughput, robustness, and reproducibility required for a successful laboratory setting. This study illustrates an automated workflow for phosphopeptide enrichment using the TitanSep Ti-IMAC or Zr-IMAC technology (2301). The automated liquid-handling stations used for bead equilibration and phosphopeptide enrichment in mass spectrometry analysis, allowing for parallel processing of up to 80 samples in less than 2 hours (including digest linear). Magnetic beads are automatically discarded even when they are too firmly bound to automatically display some level of high-throughput compatibility on a range of magnetic bead-handling stations. The automation of phosphopeptide enrichment was originally illustrated by Tape et al. in 2014, and enabling to automated phosphopeptide enrichment was recently reported by Jensen et al. in 2020. The current workflow adapts the phosphopeptide capture (PAC) method described by Jensen et al. to 2019 to automation on a Microlife™ high-performance magnetic beads.

OVERVIEW

Tissue heat inactivation & homogenization of 12 tissues from 3 replicates

SPECTRAL LIBRARY

DIA WORKFLOW

DIA PHOSPHO WORKFLOW

PAC digestion

PAC digestion

PAC digestion

500 ng peptide digest

60 samples/day DIA
FAIMS CV-45

60 samples/day DIA
FAIMS CV-45

200 samples/day DDA
FAIMS CV-45

+ FAIMS CV-45

500 ng

Phosphopeptide Enrichment

200 µg peptide

2.5 min (1:5 protein to bead ratio)

Spectral library DIA

directDIA

500 ng

1100 injections (12 tissues with 46 fractions on 2 instruments)

14 hours

35 reactions (24 hours with 3 technical replicates)

18 days

14 hours

35 reactions (24 hours with 3 technical replicates)

36 reactions (24 hours with 3 technical replicates)

FAIMS: Fast Analysis of Mixed Samples

REFERENCES


CONCLUSIONS

- We demonstrate an automated workflow for global proteome and phosphoproteome profiling suitable for a range of tissues by coupling PAC to phosphopeptide enrichment.
- Automated liquid-handling of high-throughput sample preparation as well as phosphopeptide enrichment and phosphopeptide enrichment in mass spectrometry analysis, allowing for parallel processing of up to 80 samples in less than 2 hours, allowing short gradient DIA analysis of 50 to 200 samples per day using a Proteome i LC system coupled to a ThermoFisher™ ESI-MS instrument.
- The approach can quantify up to 5000 native peptides in a short 24-26 hour gradient, allowing for fast sample turn-around within 24 hours.
- The proteome and phosphoprotein profiles of Cortex, without reduction in specificity, showing good recovery for low peptide inputs.
- We intend to further evaluate the feasibility of these new technologies in future studies to measure optimal sample coverage.


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RESULTS

Tissue heat inactivation & homogenization of 12 tissues from 3 replicates