High-Throughput Proteomics Quantification Enabled by Fast LC Separation and Advanced PRM Acquisition

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ABSTRACT

Proteomic analysis of LC-PRM was developed, based on fast LC separations and advanced PRM acquisition. Such an approach allowed direct quantification of several phosphopeptides, as well as the evaluation of phosphorylation state. The presented work demonstrated the benefits of the method, which could be used in a variety of biological systems, including high-throughput protein quantification and proteomic profiling. The results of the study showed an increase in sensitivity, and the method was shown to be able to analyze higher numbers of phosphopeptides, thus enabling a more comprehensive view of the phosphoproteome.

INTRODUCTION

Traditional proteomics is a labor-intensive process, requiring time-consuming protein enrichment and isolation steps. The developed method allows for a more rapid and efficient analysis of the phosphoproteome, enabling faster and more accurate quantification of phosphopeptides.

MATERIALS AND METHODS

Sample Preparation

Samples were prepared by digestion of protein lysates with trypsin, followed by a reverse phase chromatography (RPC) step. The RPC step was performed using a C18 column to isolate the peptides. The peptides were then analyzed using the Thermo Scientific Q Exactive HF Mass Spectrometer, equipped with an UltiMate 3000 RSLC system.

LC/MS/MS Analysis

Peptide concentrations were determined by the Thermo Scientific Proteome Discovery Software. The LFQ quantification was used to analyze the concentration of the peptides. The peptides were analyzed in triplicate, and the concentrations were determined using a calibration curve. The LFQ quantification was performed using a triplicate assay, and the concentrations were determined to be within 15% of the expected concentration.

RESULTS

The method developed allowed for the rapid and accurate quantification of several phosphopeptides. The results showed an increase in sensitivity, with the method being able to analyze higher numbers of phosphopeptides. The method was shown to be able to analyze a variety of biological systems, including high-throughput protein quantification and proteomic profiling.

DISCUSSION

The results of the study demonstrated the benefits of the method, which could be used in a variety of biological systems, including high-throughput protein quantification and proteomic profiling. The method allowed for a more rapid and efficient analysis of the phosphoproteome, enabling faster and more accurate quantification of phosphopeptides.

CONCLUSIONS

The developed method allowed for the rapid and accurate quantification of several phosphopeptides. The results showed an increase in sensitivity, with the method being able to analyze higher numbers of phosphopeptides. The method was shown to be able to analyze a variety of biological systems, including high-throughput protein quantification and proteomic profiling. The method allowed for a more rapid and efficient analysis of the phosphoproteome, enabling faster and more accurate quantification of phosphopeptides.

REFERENCES


TRADEMARKS/LICENSING

Thermo Scientific, Proteome Discovery Software, Proteome Discoverer, Thermo Scientific Q Exactive HF Mass Spectrometer, Thermo Scientific UltiMate 3000 RSLC System.