Sensitive Targeted Quantitative Proteomics Analysis Using Multiple Reaction Monitoring with a Novel ESI Interface

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Thermo Fisher TSQ interface

r2 = 0.9999

Intensity

Heated capillary

RT: 29.42

Fig. 4. LC-MRM analysis of bovine beta-lactoglobulin (DA1/DA4) in depleted human plasma using the ion funnel interface.

Calibration curve for human fibroblast growth factor 4 (FGF4; VGVQFVGVR) using the ion funnel interface.

Peak area:

Represents blanks without the spiked in peptides, i.e., endogenous level plus residual signal from the "C" channel.

Table 1. Summary of MRM results with the original Thermo and the PNNL ion funnel interfaces.

Conclusions

• With the new interface, detection of low ng/mL range of proteins in human and mouse plasma can be achieved without fractionation.

• In general, significantly improved limit of detection with the ion funnel interface.

• More reproducible measurements at lower levels with enhanced signal provided by the ion funnel interface.

Acknowledgements

We especially thank Steven Carr’s laboratory at the Broad Institute of MIT and Harvard and Amanda Paulovich’s laboratory at the Fred Hutchinson Cancer Research Center for their platform has achieved the consistent detection of proteins present at low ng/mL levels in non-fractionated blood plasma.

References


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1. Sensitive Targeted Quantitative Proteomics Analysis Using Multiple Reaction Monitoring with a Novel ESI Interface

Overview

The research presented here demonstrates significantly improved multiple reaction monitoring (MRM) performance using an in-house modified ThermoFisher TSQ instrument equipped with a novel high efficiency ESI interface incorporating a PNNL-designed dual electrodynamic ion funnel.

The modified MS/MS instrumental platform has significantly increased our ability to detect low abundant proteins in complex biological matrices.

Notably, the enhanced MS/MS instrumental platform has achieved the consistent detection of proteins present at low ng/mL levels in non-fractionated blood plasma.

Introduction

Identification and accurate quantification of low abundant proteins and peptides in complex biological samples (e.g., plasma and serum) remain challenging by the limited sensitivity, dynamic range, and quantitative precision afforded by routine "global" survey strategies.

Targeted LC-MS/MS, e.g., MRM, is increasingly being used in hypothesis-driven quantitative proteomics studies, e.g., biomarker verification.

Sensitive and selective detection of specific peptides is achieved by tailoring and monitoring both parent and fragment ions (see below).

Methods

• Targeted LC-MS/MS, e.g., MRM, is increasingly being used in hypothesis-driven quantitative proteomics studies, e.g., biomarker verification.

• Sensitive and selective detection of specific peptides is achieved by tailoring and monitoring both parent and fragment ions (see below).

• MRM detection of human plasma proteins at ng/mL and ~2 – 40 ng/mL, without fractionation has been recently reported.

Results

Fig. 3. A ThermoFisher TSQ Ultra elite quadrupole mass spectrometer equipped with an ion funnel interface incorporating dual electrodynamic ion funnels.

Fig. 4. LC-MRM analysis of bovine beta-lactoglobulin (DA1/DA4) in depleted human plasma using the ion funnel interface.

Fig. 5. Increased MRM intensity using ion funnel (1) TSQ SRM peak area for targeted standard peptides (blue) spiked into non-fractionated biological matrices (black).

Fig. 6. Calibration curve for human fibrinectin growth factor 4 (FGF4; VGVQFVGVR) using the ion funnel interface. Note: 1 fmol, represents blanks without the spiked in peptides (i.e., endogenous level plus residual signal from the "C" channel).

Fig. 7. LC-MRM analysis of OGVQYGQOR (ADAM17) spiked into depleted human plasma. Left panel: limit of detection (LOD) using the Thermo interface; middle panel: detection of the same concentration using the ion funnel interface. Inset panel: new LOD (C) funnel interface. Inset panel: detection of 1 fmol, represents blanks without the spiked in peptides, i.e., endogenous level plus residual signal from the "C" channel using the funnel interface. See Figure 8 (panel center) for OGVQYGQOR calibration curves.

Table 1. Summary of MRM results with the original Thermo and the PNNL ion funnel interfaces.