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Immunocapture LC-MS/MS Assay for the Biomarker, Troponin Fast (TNNI2) Using a Specific Antibody

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PURPOSE

One of the hallmarks of injured skeletal muscle is the appearance of elevated skeletal muscle proteins in circulation. A biomarker, Troponin Fast (TNNI2) is a surrogate to measure this muscle damage. A specific and sensitive assay for TNNI2 is necessary to understand the muscle damage in severe myopathy patients with Becker and Duchenne muscular dystrophy.
Here we report on a specific assay for the LC-MS/MS quantitative analysis of TNNI2 from human plasma.

OBJECTIVE(S)

Develop an immunoaffinity (IA) capture, HPLC-MS/MS method for the analysis of TNNI2.

METHOD(S)

The immunoaffinity extraction will be performed on human plasma samples, a well characterized highly specific mouse Anti-TNNI2 Monoclonal Antibody (mAb) clone 9C10 covalently immobilized to tosyl-activated magnetic beads will be utilized to capture TNN2. The plasma sample processing and subsequent elution will be performed on KingFisher magnetic particle processor (Thermo Fisher Scientific).

Trypsin was used to digest TNNI2 into peptides to be analyzed by LC-MS/MS. Two surrogate peptides (ELEDMNQK and MSADAMLK) were followed with MS/MS transitions 503.7 to 764.3 and 433.7 to 735.5, respectively. A 6500+ Sciex mass spectrometer was used with a 30 series UPLC and autosampler from Shimadzu. A Phenomenex Kinetex C18 (2.1x30 mm, 2.6 µm) column was used to separate the peptides. A flow rate of 0.6 mL/min was used with a gradient mobile phase mixture of acetonitrile/water and 0.1% formic acid.

RESULT(S)

To aid specific immunocapture of biomarker TNNI2 from human plasma, four mouse Anti-TNNI2 monoclonal antibody (mAb) clones were screened. Out of 4 clones, mouse Anti-TNNI2 mAb clone 9C10 was selected to be used as immunocapture reagent for having higher pM (picomolar) binding affinity and very slower dissociation rate (t ½ life > 8 days) with minimal cross reactivity to other slow skeletal muscle Troponin 1 (TNNI1). The Octet binding data indicates the IA-HPLC-MS/MS assay using mAb clone 9C10 can be employed to specifically detect TNNI2 in human plasma with minimal interference of TNNI1.

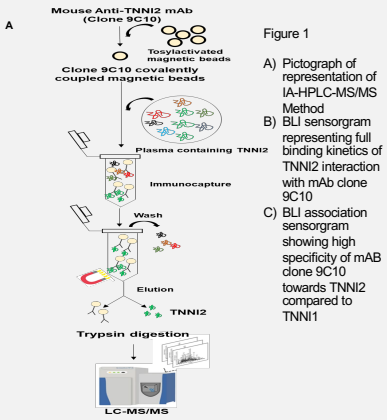

Figure 1

A) Pictograph of representation of IA-HPLC-MS/MS Method
B) BLI sensorgram representing full binding kinetics of TNNI2 interaction with mAb clone 9C10
C) BLI association sensorgram showing high specificity of mAB clone 9C10 towards TNNI2 compared to TNNI1

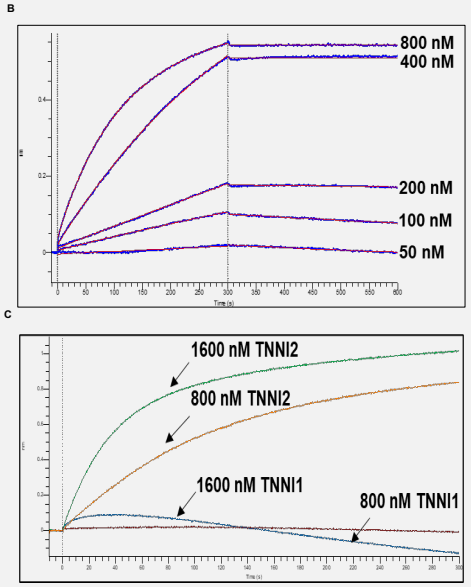

Figure 2
HPLC-MS/MS Chromatogram from the Analysis of a Digest of TNNI2 at 35 ng/mL

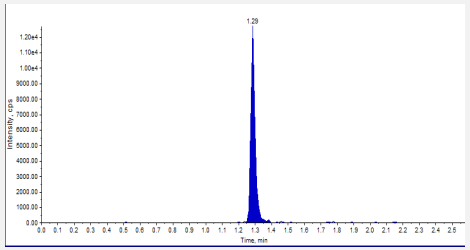

Table 1. HPLC-MS/MS Results from the Analysis of a Digestion of TNNI2

	STD-1	STD-2	STD-3	STD-4	STD-5	STD-6
Concentration	35 ng/mL	70 ng/mL	350 ng/mL	700 ng/mL	3500 ng/mL	7000 ng/mL
TNNI2 Curve	33.3	69.7	357	722	3530	6940
% Accuracy	95.1	99.6	102	103.1	100.9	99.1
n	1	1	1	1	1	1

CONCLUSION(S)

We developed a specific, sensitive, accurate and precise method for the HPLC-MS/MS analysis of TNNI2 from human plasma. This method will be used for upcoming clinical studies to determine the efficacy of therapeutics for the treatment of Duchenne and Becker Muscular Dystrophy.

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