

ABSTRACT

Accurate and robust quantification of insulin-like growth factor 1 (IGF-1) from serum using mass spectrometry (MS) is challenging due to its high complexity and dynamic range. Low cost, high throughput enrichment method for such low abundant proteins from the serum is required to enrich target proteins prior to MS analysis. We report a high-throughput sample preparation method using reverse phase micro-solid phase extraction enrichment on Microlab®NIMBUS® robotic liquid handling system from Hamilton Robotics and demonstrated over 71% recovery from serum for 96 samples in less than 30 minutes. IGF-1 levels in serum correlates well to other published methods that involve longer processing times.

INTRODUCTION

Insulin-like growth factor 1 (IGF-1) is a polypeptide hormone that plays an import role in childhood growth and continues to have anabolic effects in adults. Accurate and robust quantification of IGF-1 from serum using mass spectrometry (MS) is challenging due to its high complexity and dynamic range. Low cost, high throughput enrichment method for suc low abundant proteins from the serum is required to enrich target proteins prior to MS anal Herein, we report a high-throughput sample preparation method using reverse phase micro-solid phase extraction enrichment on a robotic liquid handling system.

MATERIALS AND METHODS

Two peptides of IGF-1 were selected for multiple reaction monitoring and the transition conditions were optimized. 100 ng of IGF-1 was spiked in 80 µg BSA and E. coli digest and isolated using automated pipetting system (Figure 1). Three different binding buffers were us 10% acetonitrile (ACN), 2% acetic acid (Buffer A, pH 3), 10% ACN, 100 mM ammonium bicarbonate (Buffer B, pH 8), and 10% ACN, 100 mM ammonium bicarbonate, 1% ammon hydroxide (Buffer C, pH 10). 5 mg divinylbenzene (DVB) loose resin in IMCStips[™] was activated with 100% acetonitrile three times and then equilibrated with binding buffer. Samples were add to $100 \ \mu$ L binding buffer and enriched by pipetting sample 20 times. Non-specific binding proteins were removed by pipetting 150 µL binding buffer three times twice and bound IGF-1 was eluted with 70% ACN, 100 mM ammonium bicarbonate buffer Organic solvent was dried under nitrogen flow for 20 minutes and 10 mM dithiothreitol wa added for denaturing at 65 °C for 30 min. Then 25 mM iodoacetamide was added for alkyla in dark for 30 minutes followed by 2 µg trypsin addition for enzymatic digestion at 37 °C overnight.

To measure enriched IGF-1 amount, we used UPLC TSQ-Endura triple quadrupole mass spectrometry with optimized conditions. Briefly, digested peptides were separated on C18 column (ThermoFisher, Syncronis, 100 x 2.1 mm, 1.7 µm) 5% - 50% acetonitrile, 0.1% formic acid gradient for 15 minutes and 769.7 m/z (T1) and 556.6 m/z (T2) precursor ions selected for fragments of quantification (Figure 2). The area under the curve (AUC) of enric samples were compared with the equal amount of exogenous IGF-1 protein standard.

- 100 ng of human recombinant IGF-1 was spiked in 100 µg protein mixture and 20 ug rat serum, respectively
- IMCS DVB tips on automatic pipetting system
- LC-MS/MS analysis to quantitate three IGF-1 peptides
- Thermo TSQ Vanquish UPLC
- Thermo TSQ Endura

- Mobile phase A: 0.1% formic acid in wat • Mobile phase B: 0.1% formic acid in
- acetonitrile
- LC gradient: 5% B 50% B for 15min of min
- Column: ThermoFisher, Syncronis C18, x 2.1 mm, 1.7 μm
- Column oven 40 °C

RESULTS

We established targeted LC-MS/MS analysis for the human IGF-1 and heavy isotope label IGF-1 (*Table 1*). We successfully demonstrated a linear calibration curve in a range from 1 to 16 ng IGF-1 with R²=0.999 (*Figure 3*). Using DVB IMCStips with high-throughput rob system, target protein was enriched within 30 minutes. We achieved 72% recovery of the IG T2 peptide from 100 µg of protein mixture using pH 10 binding buffer (*Figure 4*). Additionally, we demonstrated the quantification of the human IGF-1 standard in rat serum with/without DVB IMCStips enrichment (Figure 5).

Dispersive Pipette Extraction for Automated Enrichment of IGF-1 from Serum

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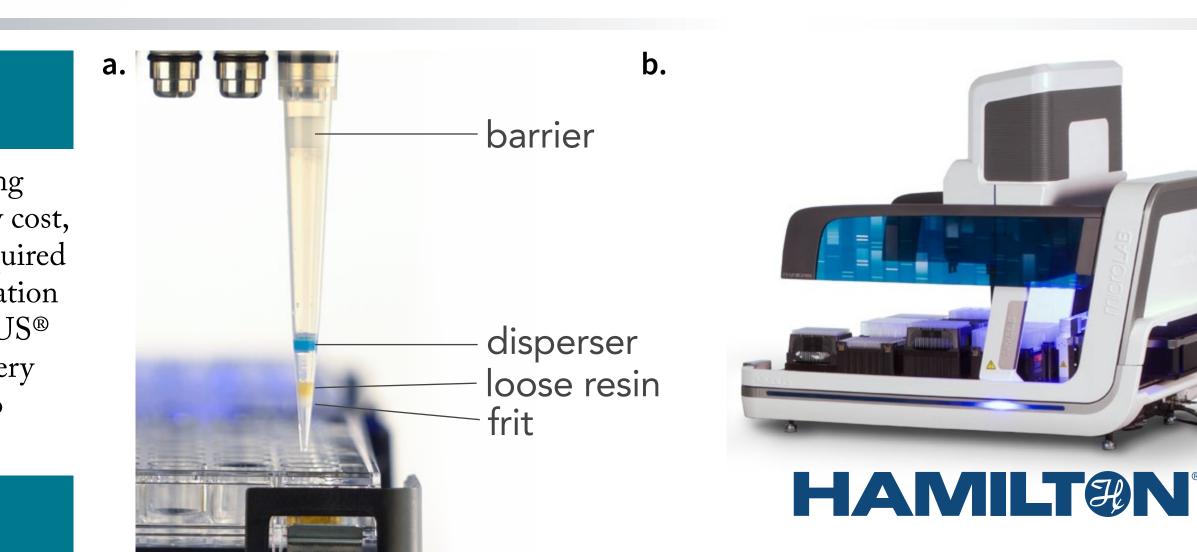
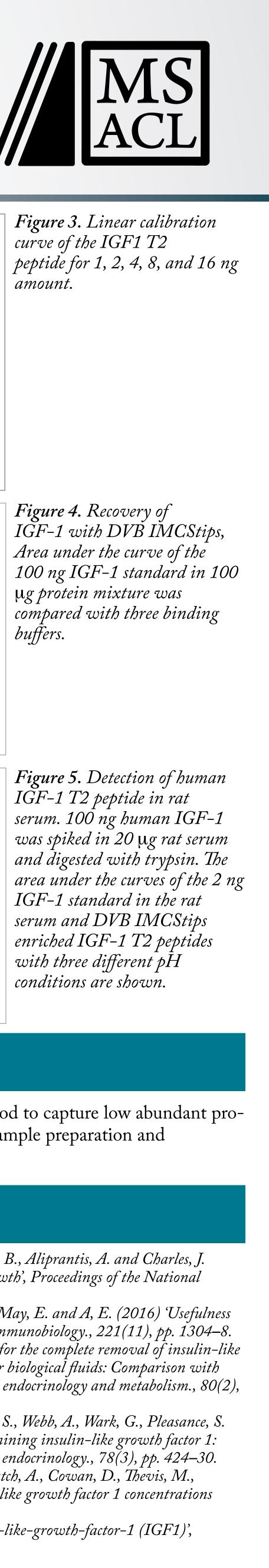


Figure 1. (a) IMCStips diagram (b) Nimbus96 automated liquid handler.

Table 1. Selected Reaction Monitoring (SRM) of IGF-1 peptides and Angiotensin II.

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$ \begin{array}{c} \text{GF1-T1} & \text{GPETLCGAELVDALQFVCGDR} & 769.696 & 507.19 & 22 \\ \text{IGF1-T1h} & \text{GPETLCGAELVDALQFVCGDR} & 773.032 & 1190.19 & 19 \\ \text{IGF1-T1h} & \text{GPETLCGAELVDALQFVCGDR} & 773.032 & 517.20 & 22 \\ \text{IGF1-T1h} & \text{GPETLCGAELVDALQFVCGDR} & 773.032 & 517.20 & 22 \\ \text{IGF1-T2} & \text{GFYFNKPTGYGSSSR} & 556.598 & 732.34 & 16 \\ \text{IGF1-T2} & \text{GFYFNKPTGYGSSSR} & 556.598 & 752.34 & 16 \\ \text{IGF1-T2} & \text{GFYFNKPTGYGSSSR} & 556.598 & 650.81 & 23 \\ \text{IGF1-T2h} & \text{GFYFNKPTGYGSSSR} & 556.598 & 650.81 & 23 \\ \text{IGF1-T2h} & \text{GFYFNKPTGYGSSSR} & 556.506 & 921.43 & 16 \\ \text{IGF1-T2h} & \text{GFYFNKPTGYGSSSR} & 556.506 & 659.82 & 23 \\ \text{Angiotensin II } & \text{DRVYIHPF} & 349.518 & 371.203 & 13 \\ \text{Angiotensin II } & \text{DRVYIHPF} & 349.518 & 513.282 & 13 \\ \end{array} $	S1S.	IGF1-T1		GPETLCGAELVDALQFVCGDR	769.696	1180.54	19	
$ \begin{array}{c} \text{IGF1-T1h} & \text{GPETLCGAELVDALQFVCGDR} & 773.032 & 1190.19 & 19 \\ \text{IGF1-T1h} & \text{GPETLCGAELVDALQFVCGDR} & 773.032 & 891.40 & 22 \\ \text{IGF1-T1h} & \text{GPETLCGAELVDALQFVCGDR} & 773.032 & 517.20 & 22 \\ \text{IGF1-T2} & \text{GFVFNNPTGYGSSSR} & 556.598 & 911.42 & 16 \\ \text{IGF1-T2} & \text{GFVFNNPTGYGSSSR} & 556.598 & 911.42 & 16 \\ \text{IGF1-T2} & \text{GFVFNNPTGYGSSSR} & 556.598 & 650.81 & 23 \\ \text{IGF1-T2h} & \text{GFVFNNPTGYGSSSR} & 556.598 & 650.81 & 23 \\ \text{IGF1-T2h} & \text{GFVFNNPTGYGSSSR} & 562.606 & 741.36 & 16 \\ \text{IGF1-T2h} & \text{GFVFNNPTGYGSSSR} & 562.606 & 659.82 & 23 \\ \text{Angiotensin II} & \text{DRVYIHPF} & 349.518 & 371.203 & 13 \\ \text{Angiotensin II} & \text{DRVYIHPF} & 349.518 & 513.282 & 13 \\ \end{array} $		IGF1-T1		GPETLCGAELVDALQFVCGDR	769.696	881.39	22	
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		IGF1-T1h		GPETLCGAELVDALQFVCGDR	773.032	891.40	22	
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	ım	IGF1-T2h		GFYFNKPTGYGSSSR	562.606	921.43	16	
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Figure 2. Total Ion Chromatogram and SRM of IGF1 and Angiotensin II, (a) Total Ion chromatogram of the 5 ng IGF1 digest and SRM transition of T2 peptide, (b) Representative extracted ion chromatogram of the enriched IGF1 using DVB IMCStips.



IGF1-T2 Calibration Curve y=2E-05x ng R²=0.99994 AUC Recovery of IGF-1 using IMCStips 400,000 350,000 Charge 300,000 62% 72% - 250,000 ں State . ₹ 200,000 150,000 100,000 50,000 IGF-1 Std. рН 8 pH 10 pH 3 Direct Inject **DVB IMCStips Enrichment** 45,000-40,000 35,000 30,000 ⊖ 25,000 20,000 15,000 10,000 5,000 IGF-1 in pH 8 pH 11 pH 3 Rat Serum

CONCLUSIONS

We successfully developed a dispersive pipette enrichment method to capture low abundant protein targets from high complex specimen for high-throughput sample preparation and analysis.

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